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Association of heavy metals with measures of pulmonary function in children and youth: Results from the National Health and Nutrition Examination Survey (NHANES)☆

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ABSTRACT

Introduction: Exposure to cadmium, cobalt, lead, and manganese has been associated with decreased pulmonary function in adults. Little is known about the magnitude of these associations among children in the United States.
Objectives: We evaluated cross-sectional associations of blood and urinary concentrations of cadmium, cobalt, lead, and manganese with pulmonary function measures [forced expiratory volume in one second (FEV1; milliliters), forced vital capacity (FVC; milliliters), ratio of FEV1 to FVC (FEV1:FVC), and mid-exhalation forced expiratory flow rate (FEF 25–75%; milliliters/second)] in a sample of 1234 6–17 year olds, who participated in the 2011–2012 survey cycle of the National Health and Nutrition Examination Survey (NHANES).
Methods: Survey-weighted linear regression was used to estimate beta coefficients and 95% confidence intervals (CIs) for the associations between metal exposure tertiles or quartiles and pulmonary function test parameters, with adjustment for relevant covariates.
Results: Blood manganese concentration was inversely associated with FVC (β for highest versus lowest quartile = −97.1, 95% CI = −230.6, 36.4; p for trend = 0.03). Urinary manganese was inversely associated with FEV1:FVC and FEF 25–75% (p for trend = 0.05 and 0.02, respectively). Urinary lead was inversely associated with FEF 25–75% (p for trend = 0.01). The associations between blood manganese and both FEV1 and FVC differed by age (p for interaction = 0.04 and 0.04, respectively), indicating an inverse trend that was strongest among older youth.
Conclusions: Environmental exposure to manganese and lead may adversely impact the pulmonary function of young people in the United States. Our findings highlight a need to prioritize children's environmental health and evaluate these associations prospectively.

1. Introduction

Exposure to heavy metals, such as cadmium, cobalt, lead, and manganese, occurs through multiple pathways, including inhalation, ingestion, and dermal absorption of contaminated air, dust, soil, food and water sources. Relative to adults, children may be particularly susceptible to inhalation exposures due to their larger lung surface area per unit body weight and increased breathing rates (Arcus-Arth and Blaisdell, 2007; Bateson and Schwartz, 2008; Poets et al., 1993). Environmental tobacco smoke is a major source of cadmium exposure, followed by emissions from mining, smelting, fuel combustion, waste incineration, and metal recycling (Agency for Toxic Substances and Disease Registry (ATSDR), 2012b). Cobalt exposure may occur due to close proximity to sites of industries which process scrap metal (Agency for Toxic Substances and Disease Registry (ATSDR), 2004). Lead exposure among children may occur through contact with contaminated soil or water and/or as a result of living in older housing with deteriorated lead-based paint (Agency for Toxic Substances and Disease Registry (ATSDR), 2007). Proximity to sites of industries which produce steel, aluminum alloys, pigments, and batteries may be sources of manganese inhalation exposure from emissions (Agency for Toxic Substances and Disease Registry (ATSDR), 2012a).

There is evidence that exposure to cadmium, cobalt, lead, and manganese may be related to a reduction in expiratory air flow rates,

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including a decrease in forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1:FVC ratio, and mid exhalation forced expiratory flow rate (FEF 25–75%) in workers with high exposure levels (Bagci et al., 2004; Boojar and Goodarzi, 2002; Jakubowski et al., 2004; Jurdziak et al., 2015; Nemery et al., 1992). In cadmium battery plant workers, blood cadmium concentrations in the highest quartile were associated with decreased values of FEV1 and peak expiratory flow rates relative to those in the lowest exposure group (Jakubowski et al., 2004). Battery and exhaust workers exposed to lead had higher blood lead levels relative to controls, and blood lead levels were inversely associated with FEV1:FVC and FEF 25–75% (Bagci et al., 2004). Blood lead levels in another occupational study were inversely associated with FVC and FEV1 (Jurdziak et al., 2015). In a study of miners, manganese concentrations in the hair, blood, and urine of mining workers were higher than those in controls, and workers exposed to manganese experienced a decrease in FVC and FEV1 over time (Boojar and Goodarzi, 2002). A study of cobalt-exposed workers found lower levels FVC and FEV1 among those with the highest exposure level compared to lower exposure levels (Nemery et al., 1992). However, in another study, urine cobalt concentration was inversely associated with FEV1 only in workers who smoked (Verougstraete et al., 2004). Higher values for pulmonary function tests are indicative of better function; therefore, inverse associations suggest that these exposures are harmful.

Performing pulmonary function testing in children can be challenging, and the reliability and consistency of test results may be limited. Interpretation of findings must take factors related to lung growth into account when examining associations between harmful exposures and pulmonary function. Evaluations of these associations at lower exposure levels and among children have yielded mixed results. A study using the NHANES II data showed inverse associations between annual concentrations of air particulates and FVC, FEV1, and peak expiratory flow among 6 to 24 year olds in the United States, but did not have information on specific metal concentrations (Schwartz, 1989). Hong and colleagues did not observe an association with ambient levels of particulate air pollutants < 10 μm, but observed inverse associations for concentrations of manganese and lead measured from the collected PM_{10} with peak expiratory flow rate among 43 school children in Korea (Hong et al., 2007). A comparison of children living in an e-waste disposal and recycling area in China to children living in unexposed areas in China showed that children differed by socioeconomic and growth factors, but no association was observed between blood concentrations of cadmium or lead with FVC or FEV1 despite blood lead levels being significantly higher in children living in the high exposure area (Zeng et al., 2017). Other studies in children have not observed any associations between urine cadmium levels and FEV1, FVC, or FEF 25–75% (Leung et al., 2013), blood lead levels and asthma (Wells et al., 2014), or blood manganese concentrations and FVC (Zheng et al., 2013). These associations have not been well-characterized among children in the United States, despite the existence of objective measures of exposure and pulmonary function.

The objective of this study is to examine the associations of blood and urinary cadmium, cobalt, lead, and manganese concentrations with pulmonary function in a nationally representative sample of children and adolescents in the United States, who participated in the 2011–2012 survey cycle of the National Health and Nutrition Examination Survey (NHANES). We also evaluated sex and age as potential effect modifiers of these associations.

2. Methods

2.1. Study population

The National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention conducts the NHANES using a complex sampling frame to attain a sample representative of the United States population. The NHANES study protocols are approved by the Institutional Review Board of the NCHS (National Center for Health Statistics, 2017), and written informed consent and/or assent is obtained from participants. We obtained publicly available data for 9756 participants assessed during the 2011–2012 survey cycle. A total of 2206 participants were between the ages of 6 to 17 years. Excluded were individuals missing data on measures of heavy metals in blood, spirometry measures, serum cotinine, family income to poverty ratio, or height. The final analytic sample consisted of 1234 observations for blood measurements and 408 observations for urine measurements, with complete data on the outcomes and covariates of interest.

2.2. Outcome measurements

Participants aged 6 and older were eligible for the spirometry component of the NHANES. Specific exclusion criteria used for the spirometry component included chest pain at the time of the exam, recent surgery of the eye, chest or abdomen, tuberculosis exposure, or a physical problem with forceful expiration (Centers for Disease Control and Prevention, 2011). Anyone with a recent incident of cough with blood or painful ear infections was also excluded. Spirometry was performed in the standing position using a standardized protocol according to the recommendations of the American Thoracic Society (ATS) (Miller et al., 2005). Participants unable to stand were allowed to complete the test from a seated position. Due to age-related differences in elastic recoil, participants aged 6 to 10 years were asked to exhale for a minimum of 3 s, while those aged 11 years and older were asked to exhale for a minimum of 6 s. Participants repeated the test for a maximum of eight attempts or until they could achieve an acceptable and reproducible spirogram. The three best spirometry readings were rated using ATS criteria (Miller et al., 2005) and recorded for each participant. Quality ratings were provided for FEV1 and FVC, and we restricted our analysis to only include spirometry data from participants with FEV1 and FVC values rated A (exceeds ATS data collection standards: 3 acceptable curves present and 2 reproducible curves; 2 observed values within 100 ml) or B (meets ATS data collection standards: 3 acceptable curves present and 2 reproducible curves; 2 observed values within 150 ml). The spirometry dataset contained raw values of the highest overall value of FVC estimated in milliliters, FEV1 in milliliters, and mid-exhalation forced expiratory flow rate (FEF 25–75%) in milliliters per second from reproducible curves that met or exceeded ATS standards. The ratio of FEV1 to FVC was derived by dividing FEV1 by FVC.

2.3. Exposure measurements

Participants aged 6 years and older from a one-third subsample (subsample A) were eligible for urine measurements. Cadmium (micrograms per liter), cobalt (micrograms per liter), lead (micrograms per liter), and manganese (micrograms per liter) concentrations were measured from spot urine samples using inductively coupled plasma mass spectrometry at the Division of Laboratory Sciences within the Centers for Disease Control and Prevention National Center for Environmental Health (National Center for Health Statistics, 2017). For urine measurements, the limits of detection for cadmium, cobalt, lead, and manganese were 0.056 μg/l, 0.048 μg/l, 0.08 μg/l, and 0.08 μg/l, respectively. Any measure below the limit of detection was set by NHANES as the limit of detection divided by the square root of two. Values below the limit of detection were set for cadmium (n = 205, 50.2%), cobalt (n = 2, 0.49%), lead (n = 23, 5.6%), and manganese (n = 110, 26.9%).

Whole blood was collected from participants aged 1 year and older. Cadmium (micrograms per liter), lead (micrograms per deciliter), and manganese (micrograms per liter) concentrations were measured in whole blood using inductively coupled plasma mass spectrometry. For blood measurements, the limits of detection for cadmium, lead, and manganese were 0.16 μg/l, 0.25 μg/dl, and 1.06 μg/l, respectively. Any

measure below the limit of detection was set by NHANES as the limit of detection divided by the square root of two. Values below the limit of detection were set for cadmium (n = 728, 60.5%) and lead (n = 36, 2.9%). All of the blood samples were above the detection limit for manganese.

Exposures were categorized into weighted quartiles based on the distribution of measured blood or urine concentration. Due to the high proportion of samples with cadmium levels below the limit of detection for both blood and urinary concentrations, cadmium was categorized into weighted tertiles, with the lowest exposure tertile consisting only of the participants with values below the limit of detection. Exposure tertiles/quartiles based on each exposure distribution in the study sample were modeled to evaluate potential non-linear associations in relation to the endpoints of interest. The lowest exposure category was considered the reference group in all categorical analyses.

2.4. Covariates

Participant information on age (continuous; years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, or other/multiracial), sex (male/female), and family income was self-reported via interview questionnaires. Family income was used to calculate the ratio of family income to poverty using the Department of Health and Human Services (HHS) poverty guidelines. The ratio was calculated by dividing family income by the poverty guidelines specific to the survey year. Serum cotinine levels were used to account for exposure to environmental tobacco smoke. Cotinine levels (nanograms per milliliter) were measured using an isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry method. Standing height was measured in centimeters, and weight (kilograms) was measured using a digital weight scale with the participant wearing the examination disposable shirt, pants, and slippers. Body mass index (BMI) was classified using the CDC sex-specific growth charts to categorize BMI based on age in months and years. BMI was categorized as underweight (BMI < 5th percentile), normal (BMI 5th to < 85th percentile), overweight (BMI 85th to < 95th percentile), or obese (BMI ≥ 95th percentile). Urinary creatinine (milligrams per deciliter) was measured using a Roche/Hitachi Modular P Chemistry Analyzer using an enzymatic (creatinase) method immunoassay. Use of bronchodilator (yes/no), antiasthmatic (yes/no), or inhaler (yes/no) medications in the previous 30 days was self-reported by the participant or guardian (for participants aged 6–15 years).

2.5. Statistical analysis

All analyses utilized the strata and primary sampling units (PSU) variables to account for the complex NHANES sampling design and nonresponse using Stata/IC 13.1 (College Station, TX: StataCorp LP) survey procedures. Analyses using blood measures of metals used the sample exam weights, and the urine measures used the weights for subsample A. Characteristics of participants with blood or urine measurements were compared using weighted percentages. Weighted arithmetic means and standard deviations (SD) were calculated for normally distributed outcomes (FEV1, FVC, FEV1:FVC, FEF 25–75%), by covariates and exposure quartiles (cobalt, lead, and manganese) or tertiles (cadmium).

Medians and interquartile ranges (IQR) were calculated for each exposure. The distributions of cadmium, cobalt, manganese, and lead were right skewed and were categorized for analysis. All measurements from the pulmonary function tests were modeled as continuous outcome variables. Associations were assessed using linear regression models for exposure to each heavy metal categorized into quartiles or tertiles to allow for non-linear dose responses. We also modeled each exposure as an ordinal variable to test for linear trends. All regression models included age (restricted cubic spline with four knots, continuous years), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other/multiracial), height (restricted cubic spline with four knots, centimeters), family income to poverty ratio (continuous), cotinine level (continuous), and self-reported use of an asthma inhaler or bronchodilator medication (yes/no). Additionally, urinary creatinine concentration (continuous milligrams per deciliter) was included in models of urinary metal concentrations.

Effect modification of associations was evaluated by sex and age (6 to 10 and 11 to 17 years). The protocol used for spirometry differed for children 10 and younger relative to those 11 and older; therefore we chose to dichotomize age into two groups. In addition, this cutoff point accounts for the changes in the relationship between age and lung function during the adolescent growth spurt that occurs around the ages of 10–12 (Wang et al., 1993). To evaluate effect modification, we added a cross-product term between categorized metal exposure and the effect modifier of interest in the linear regression model. A post-estimation adjusted Wald test was used to jointly evaluate all coefficients associated with each cross-product term. A p-value of 0.05 or less was interpreted as a statistically significant interaction.

A sensitivity analysis was conducted to re-examine the associations using the percent predicted values from the pulmonary function tests. This was done by repeating the linear regression modeling process used for the continuous spirometry values. For the sensitivity analysis we used the race/ethnicity, sex, age, and height of the sample participants and prediction equations to obtain percent predicted values for FEV1, FVC, FEV1:FVC, and FEF 25–75%. For 6 and 7 year old participants who identified as non-Hispanic black or non-Hispanic white, we used equations described by Wang (Wang et al., 1993), and for non-Hispanic Black, non-Hispanic white, and Mexican American participants ages 8 to 17 we used Hankinson's equations (Hankinson et al., 1999). The equations for 8 to 17 year olds were used to compute the percent predicted FEF 25–75% for 6 to 7 year olds, since a prediction equation for FEF 25–75% for this age group was not available in the Wang paper (Wang et al., 1993). The reference equations for non-Hispanic white predicted values were used with a correction factor of 0.88 to obtain values for any participants who did not have race/ethnic specific equations available (Pellegrino et al., 2005). Results using the percent predicted values as the outcome were compared with the results using the spirometry values in milliliters.

3. Results

In our sample of 1234 children and youth, the mean FEV1 was 2702.9 ml (SD 1109.5), mean FVC was 3151.9 ml (SD 1290.6), mean ratio of FEV1:FVC was 0.86 (SD 0.07) and mean FEF 25–75% was 2990.2 ml/s (SD 1354.6). The mean age of the sample was 12 years (SD 3.8) and 51% were female. Higher values for measures of pulmonary function were observed with increasing age (Table 1). FVC and FEV1 values were lower among females compared to males and differed by race/ethnicity. In bivariate analyses, measures of pulmonary function appeared to be higher with increasing serum cotinine level; however, this relationship did not persist after adjustment for age (data not shown). In general, characteristics of the 408 participants in the subsample with urine metal measurements were not appreciably different relative to those with blood measurements.

The median (IQR) for blood cadmium was 0.11 μg/l (0.08), blood lead was 0.56 μg/dl (0.37) and blood manganese was 10.0 μg/l (4.3). The median (IQR) for urinary cadmium was 0.04 μg/l (0.06), urinary cobalt was 0.46 μg/l (0.53), urinary lead was 0.29 μg/l (0.37) and urinary manganese was 0.14 μg/l (0.18).

The adjusted effect estimates for the associations of metal exposures in relation to pulmonary function are presented in Table 2. In fully adjusted models, blood manganese concentrations were associated with an inverse trend in FVC (p for trend = 0.03), with participants in the third and fourth highest quartiles of exposure having lower FVC

Table 1
Weighted characteristics of sample participants ages 6–17 in NHANES 2011–2012 with blood measurements, overall and by outcome.

Characteristic	Overall	FEV1 (ml)	FVC (ml)	FEV1:FVC	FEF 25–75% (ml/s)
	n (%)	Mean ± SD			
Age (years)					
6–9	410 (26.7)	1641.7 ± 452.9	1924.1 ± 542.5	0.86 ± 0.08	1853.3 ± 682.9
10–13	430 (34.0)	2591.5 ± 635.6	3039.6 ± 746.6	0.85 ± 0.07	2839.6 ± 939.4
14–17	394 (39.3)	3522.1 ± 776.8	4085.4 ± 926.2	0.86 ± 0.06	3894.9 ± 1056.3
Sex					
Female	612 (51.0)	2566.8 ± 869.7	2954.0 ± 1010.4	0.87 ± 0.06	2957.1 ± 1159.9
Male	622 (49.0)	2844.6 ± 1303.2	3358.1 ± 1503.2	0.85 ± 0.07	3024.6 ± 1539.1
Race/ethnicity					
White (non-Hispanic)	305 (57.5)	2803.8 ± 741.3	3288.5 ± 868.9	0.85 ± 0.04	3038.2 ± 872.6
Mexican American	237 (14.1)	2688.1 ± 1228.6	3101.5 ± 1390.6	0.87 ± 0.09	3066.2 ± 1644.5
Black (non-Hispanic)	355 (13.8)	2508.4 ± 1535.2	2911.9 ± 1756.0	0.86 ± 0.11	2864.0 ± 2063.9
Other race/multiracial	205 (7.7)	2547.7 ± 1612.3	2926.3 ± 1834.8	0.87 ± 0.11	2919.6 ± 2013.3
Other Hispanic	132 (6.9)	2453.4 ± 1234.0	2847.7 ± 1438.7	0.86 ± 0.08	2765.5 ± 1489.8
Body mass index (BMI)[a]					
Underweight	30 (2.2)	2221.0 ± 785.2	2491.9 ± 856.7	0.89 ± 0.06	2699.2 ± 1059.1
Normal weight	749 (61.7)	2603.1 ± 1061.9	3010.2 ± 1217.9	0.86 ± 0.07	2919.0 ± 1323.3
Overweight	197 (16.2)	2868.3 ± 1186.0	3353.5 ± 1347.4	0.85 ± 0.07	3138.4 ± 1434.2
Obese	258 (19.9)	2930.5 ± 1138.3	3499.9 ± 1363.0	0.84 ± 0.07	3122.4 ± 1378.7
Serum cotinine level					
Below LOD	398 (36.4)	2582.9 ± 883.1	3025.9 ± 1047.9	0.86 ± 0.07	2841.1 ± 1110.6
< 1 ng/ml	707 (53.1)	2728.3 ± 1207.3	3170.5 ± 1402.8	0.86 ± 0.07	3037.9 ± 1461.0
≥1 ng/ml	129 (10.5)	2989.9 ± 1284.6	3494.7 ± 1449.7	0.85 ± 0.07	3265.6 ± 1553.8
Blood metal concentrations: range					
Cd T1: 0.11	728 (60.5)	2588.3 ± 1094.4	3036.4 ± 1292.0	0.85 ± 0.07	2834.6 ± 1303.0
Cd T2: 0.16–0.17	102 (9.4)	2795.3 ± 971.0	3198.9 ± 1085.8	0.87 ± 0.07	3279.7 ± 1287.9
Cd T3: 0.18–2.1	404 (30.1)	2904.1 ± 1139.2	3369.3 ± 1309.1	0.86 ± 0.07	3211.9 ± 1415.1
Pb Q1: 0.18–0.44	315 (29.3)	2849.8 ± 897.6	3311.7 ± 1059.6	0.86 ± 0.06	3197.0 ± 1154.8
Pb Q2: 0.45–0.61	301 (27.3)	2851.1 ± 1021.9	3319.9 ± 1174.5	0.86 ± 0.07	3160.2 ± 1319.2
Pb Q3: 0.62–0.85	308 (22.7)	2546.9 ± 1199.4	2971.4 ± 1378.8	0.86 ± 0.07	2801.7 ± 1414.6
Pb Q4: 0.86–8.75	310 (20.7)	2469.6 ± 1337.4	2901.2 ± 1582.2	0.85 ± 0.08	2678.9 ± 1485.7
Mn Q1: 3.75–8.22	308 (24.3)	2775.4 ± 1242.8	3264.4 ± 1441.7	0.85 ± 0.07	2980.4 ± 1419.7
Mn Q2: 8.23–10.01	309 (25.0)	2767.0 ± 1221.4	3243.0 ± 1412.6	0.85 ± 0.07	3009.0 ± 1486.0
Mn Q3: 10.02–12.34	309 (23.8)	2607.2 ± 1023.1	3013.1 ± 1192.8	0.87 ± 0.07	2976.7 ± 1311.2
Mn Q4: 12.35–58.86	308 (26.9)	2662.4 ± 929.0	3088.4 ± 1083.2	0.86 ± 0.07	2993.4 ± 1201.0
Urine metal concentrations: range					
Cd T1: 0.04	205 (53.2)	2481.0 ± 1016.9	2931.6 ± 1204.3	0.85 ± 0.08	2670.4 ± 1235.5
Cd T2: 0.06–0.09	68 (18.3)	2680.6 ± 1043.3	3163.2 ± 1251.3	0.85 ± 0.08	2922.9 ± 1315.8
Cd T3: 0.10–0.80	135 (28.5)	2906.7 ± 1153.3	3408.6 ± 1358.6	0.86 ± 0.09	3169.6 ± 1513.9
Pb Q1: 0.06–0.17	99 (28.8)	2677.5 ± 988.1	3123.7 ± 1164.5	0.86 ± 0.08	2996.8 ± 1282.4
Pb Q2: 0.18–0.32	102 (26.0)	2769.2 ± 960.7	3267.2 ± 1139.2	0.85 ± 0.09	3008.5 ± 1281.3
Pb Q3: 0.33–0.56	105 (24.2)	2665.6 ± 1299.3	3175.2 ± 1528.6	0.84 ± 0.08	2756.4 ± 1507.9
Pb Q4: 0.57–5.12	102 (21.0)	2395.2 ± 1025.8	2823.1 ± 1228.8	0.85 ± 0.07	2604.2 ± 1254.5
Mn Q1: 0.06	110 (29.1)	2724.5 ± 1038.2	3174.4 ± 1210.3	0.86 ± 0.08	3047.3 ± 1361.0
Mn Q2: 0.08–0.14	91 (22.0)	2834.4 ± 1151.8	3336.2 ± 1332.7	0.85 ± 0.08	3056.7 ± 1472.4
Mn Q3: 0.15–0.24	109 (24.0)	2553.6 ± 1093.3	3027.5 ± 1392.3	0.85 ± 0.08	2696.0 ± 1181.6
Mn Q4: 0.25–2.73	98 (24.9)	2448.5 ± 1009.0	2914.6 ± 1164.8	0.84 ± 0.08	2621.5 ± 1290.0
Co Q1: 0.03–0.23	101 (24.7)	2551.7 ± 1023.7	2957.5 ± 1163.4	0.86 ± 0.08	2907.7 ± 1426.6
Co Q2: 0.24–0.43	103 (22.4)	2641.4 ± 1249.1	3162.0 ± 1488.2	0.84 ± 0.09	2774.3 ± 1535.1
Co Q3: 0.44–0.68	102 (24.6)	2610.1 ± 1160.0	3099.7 ± 1406.8	0.85 ± 0.08	2735.0 ± 1312.0
Co Q4: 0.69–3.5	102 (28.3)	2738.1 ± 930.0	3210.7 ± 1092.1	0.85 ± 0.06	2991.4 ± 1169.3

Notes: FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FEV1:FVC, ratio of FEV1 to FVC; FEF 25–75%, mid exhalation forced expiratory flow rate; LOD, limit of detection; Cd, cadmium; Pb, lead; Mn, manganese; Co, cobalt.

[a] NHANES classified participants as underweight (BMI < 5th percentile), normal weight (BMI 5th to < 85th percentile), overweight (BMI 85th to < 95th percentile), or obese (BMI ≥ 95th percentile).

measures relative to participants in the lowest exposure quartile. Urinary manganese concentrations were associated with inverse trends for the ratio of FEV1:FVC and FEF 25–75% (p for trend = 0.05 and 0.03, respectively). Urinary lead concentrations were associated with inverse trends for FEF 25–75% (p for trend = 0.02). Modeling creatinine using a log transformation or cubic spline (relative to an untransformed continuous variable) in the fully adjusted urinary models did not appreciably change the effect estimates or associations.

We observed significant interactions between age and blood concentrations of manganese in relation to FEV1 and FVC (p for interaction = 0.04 and 0.04, respectively). As shown in Figs. 1 and 2, an inverse association was observed among older youth aged 11–17 years (p for trend = 0.05 and 0.05 for FEV1 and FVC, respectively), but was not observed among younger participants. A significant interaction was observed between sex and urine cadmium concentration in relation to FEF 25–75% (p for interaction = 0.007), suggesting that males are more susceptible to the effects of cadmium exposure. However, the p value for trend in neither sex was significant (p = 0.44 for males and p = 0.12 for females).

In our sensitivity analyses (not shown), we did not observe a strong trend between blood manganese concentrations and percent predicted FVC (p for trend = 0.16). The inverse association persisted between urinary manganese concentration and percent predicted FEF 25–75% (β for highest versus lowest quartile = −10.3, 95% CI = −19.2, −1.4; p for trend = 0.04), and was marginally associated with the percent predicted ratio of FEV1:FVC (β for highest versus lowest

Table 2
Multivariable adjusted[a] associations between metal exposure quartiles or tertiles and pulmonary function test parameters in sample participants aged 6–17 years in the NHANES 2011–2012 survey cycle (n = 1234 blood; n = 408 urine).

	n	FEV 1 (ml)	FVC (ml)	FEV1:FVC	FEF 25–75% (ml/s)
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Blood concentrations					
Cadmium (μg/l)					
T1 (0.11)	728	Ref	Ref	Ref	Ref
T2 (0.16–0.17)	102	−4.0 (−171.2, 163.2)	−67.7 (−268.3, 132.9)	0.01 (−0.009, 0.04)	184.1 (−112.1, 480.4)
T3 (0.18–2.1)	404	5.2 (−66.3, 77.7)	−11.7 (−108.4, 84.9)	0.002 (−0.004, 0.009)	12.0 (−66.6, 90.6)
p for trend		0.88	0.72	0.30	0.49
Lead (μg/dl)					
Q1 (0.18–0.44)	315	Ref	Ref	Ref	Ref
Q2 (0.45–0.61)	301	4.8 (−98.3, 107.8)	1.6 (−88.5, 91.7)	0.0003 (−0.01, 0.01)	−8.1 (−229.8, 213.7)
Q3 (0.62–0.85)	308	22.3 (−49.3, 93.9)	23.8 (−46.4, 94.0)	−0.001 (−0.01, 0.01)	−28.9 (−160.5, 102.7)
Q4 (0.86–8.75)	310	41.9 (−46.9, 130.6)	45.5 (−49.2, 140.2)	0.002 (−0.01, 0.02)	0.71 (−193.1, 192.5)
p for trend		0.32	0.30	0.78	0.91
Manganese (μg/l)					
Q1 (3.75–8.22)	308	Ref	Ref	Ref	Ref
Q2 (8.23–10.01)	309	45.3 (−53.4, 144.1)	37.1 (−75.0, 149.2)	0.004 (−0.009, 0.02)	99.6 (−53.2, 252.4)
Q3 (10.02–12.34)	309	−39.3 (−139.7, 61.1)	−101.1 (−193.9, −8.2)	0.02 (0.003, 0.03)	134.2 (−52.7, 321.2)
Q4 (12.35–58.86)	308	−51.5 (−176.0, 73.0)	−97.1 (−230.6, 36.4)	0.01 (−0.005, 0.03)	69.0 (−159.0, 297.1)
p for trend		0.19	0.03	0.07	0.50
Urinary concentrations					
Cadmium (μg/l)					
T1 (0.04)	205	Ref	Ref	Ref	Ref
T2 (0.06–0.09)	68	54.4 (−75.2, 184.1)	55.0 (−124.9, 234.9)	0.004 (−0.02, 0.03)	117.2 (−144.1, 378.5)
T3 (0.10–0.80)	135	−31.3 (−149.0, 86.5)	−42.9 (−181.3, 95.5)	0.003 (−0.01, 0.02)	−2.6 (−254.2, 249.0)
p for trend		0.77	0.70	0.68	0.85
Cobalt (μg/l)					
Q1 (0.03–0.23)	101	Ref	Ref	Ref	Ref
Q2 (0.24–0.43)	103	−87.7 (−233.4, 58.0)	−13.5 (−133.7, 106.7)	−0.02 (−0.05, 0.006)	−289.4 (−670.6, 91.8)
Q3 (0.44–0.68)	102	−27.9 (−153.4, 97.5)	32.3 (−123.4, 188.0)	−0.01 (−0.03, 0.007)	−238.6 (−461.2, −16.0)
Q4 (0.69–3.5)	102	−62.8 (−211.7, 86.2)	−21.5 (−177.2, 134.2)	−0.01 (−0.03, 0.01)	−193.1 (−552.3, 166.2)
p for trend		0.54	0.89	0.49	0.27
Lead (μg/l)					
Q1 (0.06–0.17)	99	Ref	Ref	Ref	Ref
Q2 (0.18–0.32)	102	−28.3 (−199.2, 142.6)	36.5 (−97.4, 170.4)	−0.02 (−0.05, 0.01)	−191.1 (−571.0, 188.9)
Q3 (0.33–0.56)	105	−87.1 (−259.0, 84.8)	−27.9 (−235.4, 179.5)	−0.02 (−0.04, 0.002)	−351.2 (−608.7, −93.7)
Q4 (0.57–5.12)	102	−96.5 (−307.9, 114.9)	−30.9 (−285.4, 223.4)	−0.02 (−0.05, 0.006)	−315.8 (−708.0, 76.3)
p for trend		0.24	0.66	0.12	0.01
Manganese (μg/l)					
Q1 (0.06)	110	Ref	Ref	Ref	Ref
Q2 (0.08–0.14)	91	2.7 (−148.6, 154.1)	48.5 (−104.8, 201.8)	−0.01 (−0.04, 0.01)	−147.9 (−490.7, 194.9)
Q3 (0.15–0.24)	109	−36.9 (−214.4, 140.6)	23.4 (−149.2, 195.9)	−0.01 (−0.05, 0.02)	−235.5 (−578.4, 107.5)
Q4 (0.25–2.73)	98	−86.3 (−238.9, 66.3)	−3.6 (−202.1, 194.9)	−0.03 (−0.05, −0.004)	−290.0 (−554.6, −25.5)
p for trend		0.25	0.97	0.05	0.02

[a] Model is adjusted for age (cubic spline), sex (male/female), race (non-Hispanic black, non-Hispanic white, Mexican American, other Hispanic, other/multiracial), height (cubic spline), family income to poverty ratio (continuous), serum cotinine (continuous), use of antiasthmatic, bronchodilator, or inhaler medications (yes/no), urine creatinine (continuous; urinary concentration models only).

quartile = −3.1, 95% CI = −6.1, −0.06; p for trend = 0.07). Urinary lead concentrations remained associated with an inverse trend for percent predicted FEF 25–75% (β for highest versus lowest quartile = −7.1, 95% CI = −17.5, 3.3; p for trend = 0.03). In general, results from interaction analyses using pulmonary function parameters in milliliters were not appreciably different relative to those using percent predicted values (data not shown).

4. Discussion

In this study, we observed inverse associations between blood and urinary concentrations of manganese and urinary concentrations of lead with multiple parameters of pulmonary function tests in children and adolescents in the United States. We also observed that the associations between blood concentrations of manganese and measures of FEV1 and FVC were strongest among older children. Although the interpretation of spirometry results in children differs from adults (Seed et al., 2012; Stocks et al., 2014), with optimal participant cooperation quality measurements of FEV1 and FVC can be obtained from children in settings where specially trained technicians are available (Beydon et al., 2007). In children and youth, FEV1 is considered an appropriate measure of large airway obstruction, and may be the best measure of pulmonary function in young children. FVC is dependent on forced expiratory time, and may be less reliable in children due to the difficulty in blowing to residual volume. Although ATS guidelines do not indicate FEF 25–75% as an indicator of airway obstruction, it may be a useful tool to understand small airway patency and inflammation (Chiang and Hsu, 1997; Lebecque et al., 1993; Piccioni et al., 2015; Rao et al., 2012). In adults, generally accepted guidelines for pulmonary function testing use a 200 ml change in parameters of pulmonary function to evaluate the usefulness of medications to improve pulmonary function (Pellegrino et al., 2005). Although such a large change cannot be expected in children, the magnitude of the inverse associations we have observed may be clinically meaningful, and if true, indicate that increased exposure to manganese and lead may contribute to impairments in pulmonary function among young people during a vital period of growth and development. To the best of our knowledge, this is the first study to investigate these associations using continuous pulmonary function parameters in a nationally representative sample of children and adolescents in the United States.

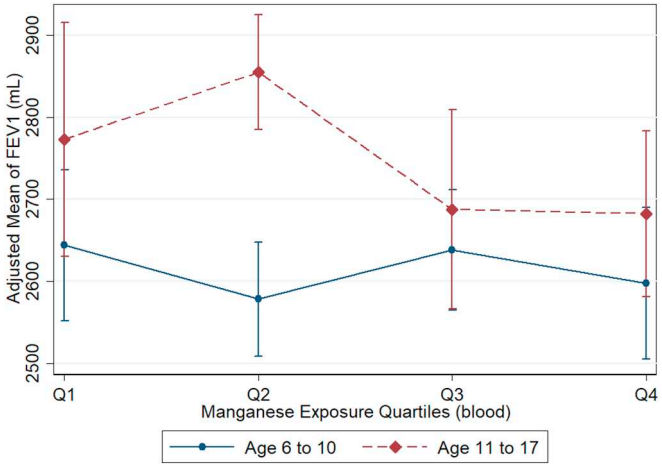

Fig. 1. Multivariable adjusted age specific mean estimates of FEV1 by manganese exposure quartiles in sample participants (n = 1234). Models were adjusted for sex (male/female), race (non-Hispanic black, non-Hispanic white, Mexican American, other Hispanic, other/multiracial), height (cubic spline), family income to poverty ratio (continuous), serum cotinine (continuous), use of antiasthmatic, bronchodilator, or inhaler medications (yes/no). Error bars represent 95% CIs.

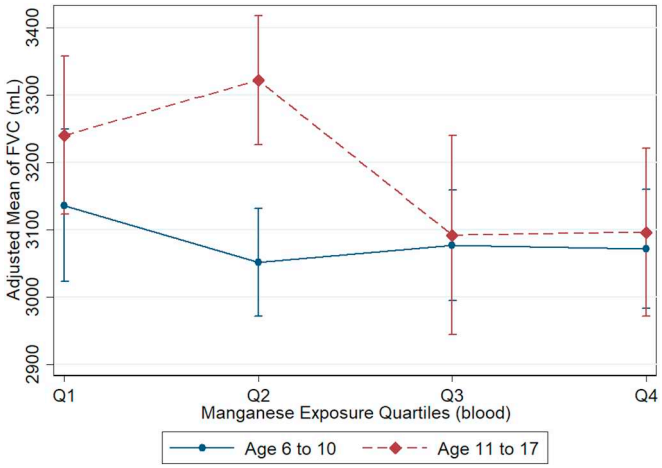

Fig. 2. Multivariable adjusted age specific mean estimates of FVC by manganese exposure quartiles in sample participants (n = 1234). Models were adjusted for sex (male/female), race (non-Hispanic black, non-Hispanic white, Mexican American, other Hispanic, other/multiracial), height (cubic spline), family income to poverty ratio (continuous), serum cotinine (continuous), use of antiasthmatic, bronchodilator, or inhaler medications (yes/no). Error bars represent 95% CIs.

Previous studies evaluating associations of pulmonary function tests and respiratory symptoms in relation to manganese exposure in non-occupationally exposed populations have been inconsistent. Many studies have used measures of manganese from ambient air, and have not investigated individual-level biomarker measurements. In a study of 21 college students relocating from a rural to a highly polluted region in China, manganese levels in particulate matter were not consistently associated with pulmonary function abnormalities (Wu et al., 2013). A small study of school children in China observed lower measures of FVC among children exposed to manganese compared to unexposed children, but after controlling for height the association was no longer significant (Zheng et al., 2013). In a panel study of 43 Korean school children followed for 42 days, peak expiratory flow rates were significantly lower one day after exposure to respirable manganese and lead in particulate matter (Hong et al., 2007). No studies in children or young adults have assessed the association between manganese exposure and measures of mid-flow rate, such as FEF 25–75%. This may be because diagnosis of obstructive lung disease or asthma is difficult using FEF 25–75% alone, although mid-flow rate may be a useful measure of airway dysfunction in children with asthma with normal FEV1 values (Simon et al., 2010). Differences across these studies and our study may be related to variation in study design, age of the participants, levels of exposure, pollutant mixtures, and the use of different measures of pulmonary function in those with or without a diagnosis of asthma (Hong et al., 2007; Wu et al., 2013; Zheng et al., 2013).

Associations with blood manganese were observed in relation to FEV1 and FVC, while association with urinary manganese was only observed with FEF 25–75%. Children are likely exposed at a much lower level than in occupational settings, suggesting the relationship with pulmonary function observed in the current study is related to chronic, low-dose exposures. Chronic, low-dose exposure could explain the difference in FEV1 and FVC we observed between younger and older children, as the younger children have not been exposed for as long. Nonetheless, the selection of a reliable biomarker for assessment of manganese exposure is subject to debate (Zheng et al., 2011). Manganese measurements in blood and urine are thought to have a short half-life, and some have questioned the utility of these biomarkers in health studies (Apostoli et al., 2000). However, in animal studies an elevation in manganese concentration in whole blood following manganese inhalation has been demonstrated (Tapin et al., 2006). Human studies have found blood concentrations to be correlated with air exposure for periods as long as 90 days (Baker et al., 2016; Boojar and Goodarzi, 2002; Smith et al., 2007). This suggests that blood concentrations may reflect a consistent exposure. Discordance between workplace exposure levels and urinary concentrations of manganese has led to urinary concentrations being regarded as a less reliable biomarker of exposure (Myers et al., 2003; Smith et al., 2007). Only a small percentage of manganese is eliminated in urine, as the primary pathway for manganese elimination is through biliary excretion. This is consistent with, and may explain why, 26.9% of the urinary manganese measurements were below the limit of the detection.

The precise mechanisms by which manganese could affect pulmonary function are not known. Several have postulated increased oxidative stress as a possible mechanism (Frick et al., 2011; Hong et al., 2007; Zheng et al., 2013). Animal experiments have shown increased lung inflammation, chemokines, vascular endothelial growth factor, and decreased number of CD4 and CD8 lymphocytes (Antonini et al., 2012; Han et al., 2009) with production of inflammatory cytokines IL-6 and IL-8 and caspase-9-mediated apoptosis in human bronchial epithelial cells (Han et al., 2009; Zhang et al., 2013). Exposure to high concentrations of manganese over time may contribute to inflammation of the lung and subsequent impairment of lung function.

Studies of the association between lead and pulmonary function in children have primarily focused on diagnosed asthma, and studies investigating an association using pulmonary function tests are scarce. One study in Poland found blood lead levels to be inversely associated with FVC in children aged 10–15 years old (Little et al., 2017). A study using Missouri Medicaid claims data showed no relationship between diagnosed asthma and elevated lead levels (Rabito et al., 2013), nor was there a relationship between elevated lead levels and diagnosed asthma among children seen at a Chicago inner-city health center (Myers et al., 2002). In contrast, among 930 kindergarten students in Taiwan, lead levels $\geq 5\,\mu g/dl$ were positively associated with asthma (odds ratio = 5.5, 95% CI: 1.7 to 17.9) (Wang et al., 2017). Differences across studies could be due to variations in lead levels, endpoints investigated, or lack of adjustment for potential confounders. Lead may impact lung function by increasing tracheal responsiveness (Min et al., 2008) or through inflammatory pathways, which has been observed in animal inhalation models (Boskabady et al., 2016).

We did not observe any association between cadmium exposure and pulmonary function test parameters in our study. This is in agreement with the limited available studies which have not found an association

between urine or blood cadmium levels and FEV1, FVC, or peak expiratory flow rate (Leung et al., 2013; Zeng et al., 2017), although a study of children showed higher levels of cadmium in the hair of children with recurrent wheezing compared to healthy children (Razi et al., 2012). In our sample, 58.6% and 50.2% of participants had undetectable levels of cadmium when measured in blood and urine, respectively. We observed a statistical interaction between urinary cadmium concentration and sex in relation to FEF 25–75%, but the trends by sex were not significant. Given the young age of our sample, it is also possible that exposure levels are not high enough or long enough in duration to observe an association with cadmium exposure.

We also did not observe any association between cobalt exposure and pulmonary function test parameters in our study. Evidence for an inverse association of cobalt with pulmonary function comes primarily from occupational studies, which have shown pulmonary function measurements that are significantly lower in those with the highest exposure levels (Nemery et al., 1992; Rehfisch et al., 2012). No studies of pulmonary function and cobalt exposure in children to date have been found.

The strengths of this study include the use of a nationally representative sample with objective measurements of both exposures and outcomes of interest, as well as important covariates such as cotinine levels. Additionally, the oversampling strategy utilized by NHANES allowed for representation of populations that are often underrepresented in biomedical research, such as Hispanic/Latino persons. The large sample available from the NHANES allowed for comparisons to be made by age and sex, although statistical power may be limited for analyses of interaction effects. A limitation to our study is the cross-sectional nature of the data, where exposures and assessment of pulmonary function was only measured at one time point. These associations should be replicated using prospective data with serial spirometry measurements in the future. Exposure to air pollution is known to decrease pulmonary function in children, and it is possible that exposure to other unmeasured air pollutants correlated to those under study but unaccounted for in this analysis could explain the decreased lung function observed in this study. Finally, we did not have access to the geographic location of participants and could not determine if they were residing in urban or rural settings, in close proximity to traffic, or in industrial areas.

5. Conclusions

In summary, our findings indicate that environmental exposure to heavy metals, specifically manganese and lead, may be inversely associated with some pulmonary function test parameters in children and young adults in the United States. If confirmed, these findings may have important implications for the protection of environmental health of young persons and should be taken into consideration as future health intervention and prospective studies are developed and evaluated.

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