Evaluation of Cumulative Lead Dose and Longitudinal Changes in Structural Magnetic Resonance Imaging in Former Organolead Workers

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Objective: We evaluated whether tibia lead was associated with longitudinal change in brain volumes and white matter lesions in male former lead workers and population-based controls in whom we have previously reported on the cognitive and structural consequences of cumulative lead dose. **Methods:** We used linear regression to identify predictors of change in brain volumes and white matter lesion grade scores, using two magnetic resonance imaging scans an average of 5 years apart. **Results:** On average, total brain volume declined almost 30 cm^3, predominantly in gray matter. Increasing age at the first magnetic resonance imaging was strongly associated with larger declines in volumes and greater increases in white matter lesion scores. Tibia lead was not associated with change in brain volumes or white matter lesion scores. **Conclusions:** In former lead workers in whom cumulative lead dose was associated with progressive declines in cognitive function decades after occupational exposure had ended, cumulative lead dose was associated with earlier persistent effects on brain structure but not with additional worsening during 5 years.

We previously reported on relations of lifetime cumulative lead dose (estimated as the concentration of lead in tibia bone by x-ray fluorescence) with cognitive function and brain structure measured by magnetic resonance imaging (MRI) in a cohort of older former workers with past exposure to organic and inorganic lead.[1] The long period between last occupational lead exposure and study follow-up (an average of 16 years at the first study visit) allows us to evaluate whether lifetime lead dose was associated with reversible, persistent, or progressive effects on cognitive function and brain structure. Distinguishing among these various effects is an essential utility of longitudinal data, which is relatively rare in occupational epidemiology studies. Understanding these relations is also directly relevant to the general population, because most older Americans were exposed to high levels of environmental lead exposure in the past and can have average tibia lead concentrations that are higher than in the former workers.[2] Past cumulative inorganic lead dose is adversely associated with cognitive function in older persons in the general population.[3,4]

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We reported that higher lifetime lead dose in these former lead workers was associated with 1) poorer cognitive test scores at cross section[5] and progressive declines in cognitive function over time[6,7]; 2) smaller brain volumes in both regions of interest (ROIs) and voxel-wise analytic approaches[8]; 3) increased prevalence and severity of white matter lesions[8]; and 4) greater decrements in cognitive function from cumulative lead dose in subjects with the apolipoprotein E ϵ4 allele (*APOE*-ϵ4).[9] In cross-sectional analyses, smaller brain volumes were associated with worse cognitive function,[10] and there was evidence that the associations of lead dose with worse cognitive function were mediated, at least in part, by changes in brain volumes.[11]

With data from a second MRI, an average of 5 years later, we now report on relations of cumulative lead dose (tibia lead levels) with longitudinal changes in brain volumes and white matter lesions to evaluate whether the effects of lead dose on brain structure are likely to be reversible, persistent, or progressive.

METHODS

Study Design and Overview

Subjects were initially recruited during two study phases between 1994 and 2003, as previously described.[8] In phase I (1994 to 1997), former employees of a chemical manufacturing plant in the eastern United States were recruited. The first MRI was obtained in phase II (2001 to 2003). During phase III (2005 to 2008), summarized herein, subjects who completed the first MRI were invited for a second MRI. All phases of the study were reviewed and approved by the Johns Hopkins Bloomberg School of Public Health Committee on Human Research and written informed consent was obtained from all participants.

Selection and Recruitment of Study Subjects

The selection, recruitment, and enrollment over time of former lead workers and community-dwelling controls without occupational lead exposure (hereafter referred to as controls) have been previously reported.[5,6,8,10,12] During phase II, first MRIs were completed on 589 of 979 (60%) former lead workers and 67 of 131 (51%) controls. All participants in earlier phases of the study were eligible for this first MRI measurement. During phase III, a second MRI was obtained from 317 of 589 (54%) former lead workers and 45 of 67 (67%) controls. Second MRIs were not obtained because of death ($N = 52$), chronic illness ($N = 44$), discomfort (eg, claustrophobia, inability to lie down) with the procedure ($N = 12$), contraindications (eg, metal foreign body in eye) to MRI scanning ($N = 16$), loss to follow-up ($N = 47$), out migration ($N = 9$), and refusal for unspecified reasons ($N = 99$).

Data Collection

Detailed data collection methods for the first two phases of the study have been previously described.[8] We describe only measures specifically used for the analysis presented herein.

Subject Interview

In phase III, the subject interview was expanded to include a number of additional study variables, similar to the one used in the Baltimore Memory Study.[13,14] Health outcomes (eg, diabetes, heart disease) were ascertained by interview response to the following question format for each condition, "Has a doctor ever told you that you had [name of condition]?" For educational attainment, information was obtained by interview on years of education, trade school, general education development, and other educational certificates using previously published methods.[14]

Tibia Lead

Tibia lead, an estimate of lifetime cumulative lead dose, was available from earlier phases of the study on all former lead workers and all but one control with two MRIs. This was measured with ^{109}Cd-induced K-shell x-ray fluorescence (μg lead per gram bone mineral) and modeled as the estimated level at the end of employment (peak tibia lead), as previously described.[5]

MRI Acquisition

For the first MRI, all subjects were imaged at the same location on the same General Electric 1.5-T Signa model as previously described.[8] Eighteen of the first MRIs were not suitable for volumetric analysis due to image quality. For the second MRI, a 3-T General Electric scanner was used. T1-weighted images were acquired using a spoiled gradient recalled sequence (echo time [TE] = 8 ms, repetition time [TR] = 21 ms, flip angle = 30°, field of view [FOV] = 24 cm). Axial proton density/T2 (TR/TE/TE2 = 2200/27/120) and fluid-attenuated inversion recovery (TR/TE/T1 = 8000/100/2000) images were also acquired for lesion grading.

Clinical MRI Review and White Matter Grading

All MRIs were reviewed to exclude urgent or emergent brain disease (subjects and their physicians were notified if present).[15] MRIs were assigned a white matter lesion grade score by a trained neuroradiologist using the Cardiovascular Health Study (CHS) 10-point (0 to 9) scale,[16,17] as previously reported,[8] allowing analysis of change in ratings.

Image Analysis

The methods to obtain regional and voxel-wise volumes, including skull stripping, segmentation, registration, and transformation to regional analysis of volumes examined in normalized space (RAVENS), were completed using previously published methods.[8,18–22] Because of the inevitable changes in scanner technology and pulse sequences, we used specialized

TABLE 1. Selected Summary Statistics for 1110 Former Lead Workers and Controls Who Participated in Any Visit of the Former Lead Worker Study, 1994–2008

Variable	Former Worker (N = 979)	Control (N = 131)	No MRI (N = 439)	One MRI (N = 309)	Two MRIs (N = 362)	P Value by MRI Status*
Age at enrollment, yr, mean (SD)	56.5 (8.0)	58.6 (7.0)	57.1 (8.0)	57.6 (8.4)	55.6 (7.1)	0.002
Age at first MRI, yr, mean (SD)	60.2 (8.1)	66.7 (6.3)	—	61.6 (8.5)	60.2 (7.8)	0.03
Employment duration, yr, mean (SD)	8.0 (9.6)	—	7.2 (9.3)	8.2 (9.8)	8.8 (9.6)	0.10
Duration since last lead exposure, yr, mean (SD)	18.5 (11.1)	—	19.6 (11.5)	19.5 (11.4)	16.9 (10.5)	0.006
Controls, N (%)	0 (0%)	131 (100%)	64 (14.6%)	22 (7.1%)	45 (12.4%)	
Enrollment year, N (%)						<0.001
P1-Y1	437 (44.6%)	113 (87.0%)	260 (59.2%)	119 (38.5%)	172 (47.5%)	
P1-Y2	218 (22.3%)	14 (11.5%)	111 (25.3%)	51 (16.5%)	71 (19.6%)	
P1-Y3	48 (4.9%)	2 (1.5%)	21 (4.8%)	10 (3.2%)	19 (5.3%)	
P2-Y5	107 (10.9%)	0 (0%)	22 (5.0%)	36 (11.7%)	49 (13.5%)	
P2-Y6	169 (17.3%)	0 (0%)	25 (5.7%)	93 (10.1%)	51 (14.1%)	
Current tibia lead, N (%)	820 (83.8%)	80 (61.1%)	264 (60.1%)	274 (88.7%)	362 (100.0%)	
Current tibia lead, μg/g, mean (SD)	14.8 (9.7)	19.0 (10.8)	16.0 (9.7)	15.5 (10.6)	14.2 (9.22)	0.06
Peak tibia lead, N (%)	795 (81.2%)	0 (0%)	248 (56.5%)	242 (78.3%)	305 (84.3%)	
Peak tibia lead, μg/g, mean (SD)	25.0 (18.6)	—	27.6 (19.5)	26.9 (20.6)	21.4 (15.5)	<0.001
MRI P2, N (%)	589 (60.2%)	67 (51.2%)	—	294 (95.2%)	362 (100.0%)	
MRI P3, N (%)	332 (33.9%)	45 (34.4%)	—	15 (4.9%)	362 (100.0%)	
APOE genotype, N (%)						0.49
Not genotyped	97 (9.9%)	49 (37.4%)	134 (30.5%)	11 (12.3%)	1 (3.0%)	
ϵ2/2	3 (0.3%)	1 (1.2%)	1 (0.3%)	1 (0.3%)	2 (0.6%)	
ϵ2/3	90 (10.2%)	12 (14.6%)	30 (9.8%)	27 (9.1%)	45 (12.5%)	
ϵ3/3	565 (64.1%)	48 (58.5%)	209 (68.5%)	185 (62.1%)	219 (60.7%)	
ϵ2/4	26 (3.0%)	4 (4.9%)	9 (3.0%)	10 (3.4%)	11 (3.1%)	
ϵ3/4	176 (20.0%)	16 (19.5%)	48 (15.7%)	66 (22.2%)	78 (21.6%)	
ϵ4/4	22 (2.5%)	1 (1.2%)	8 (2.6%)	9 (3.0%)	6 (1.7%)	
CHS score, P2, mean (SD)	0.9 (1.5)	1.1 (1.5)	—	1.0 (1.5)	0.8 (1.4)	0.09
CHS score, P3, mean (SD)	1.9 (1.7)	2.6 (1.4)	—	1.7 (1.3)	2.0 (1.7)	0.48
TBV, MRI1 4D fit, cm^3, mean (SD)	1171.7 (100.8)	1140.8 (100.8)	—	—	1167.8 (101.1)	
TBV, MRI2 4D fit, cm^3, mean (SD)	1143.0 (99.4)	1102.5 (99.6)	—	—	1138.0 (100.2)	

Because former lead workers were enrolled over time, and tibia lead and MRIs were measured at different times, these data can be used to evaluate selection bias over time.
*Comparing those with one MRI to those with two MRIs.

image analysis methods that minimized the discontinuity between the two scans. We used the CLASSIC algorithm,[23] which uses a four-dimensional segmentation framework in which the baseline and follow-up scans are considered jointly during segmentation to minimize discrepancies between the two segmentations and better estimate longitudinal change. This algorithm has been previously validated.[23]

TABLE 2. Comparing Former Lead Workers and Controls With Two MRIs ($N = 362$) on Selected Variables From the Phase III Visit

Variable	Former Lead Workers ($N = 317$)	Controls ($N = 45$)	P value
Age, yr, mean (SD)*	64.1 (7.6)	71.9 (6.0)	<0.001
Education, high school graduate with or without additional trade school, N (%)	239 (75.4)	30 (66.7)	0.40†
White race/ethnicity, N (%)	284 (89.6)	42 (93.3)	0.43
APOE genotype, at least one ϵ4 allele, N (%)	81 (25.6)	14 (31.1)	0.48

*In Phase III.
†P value from five education group comparison.

Statistical Analysis

The purpose of the analysis was to determine whether the effect of lead on brain structure was progressive in nature, an essential task that requires longitudinal data; that is, after lead exposure, lead gains access to the blood, then to the brain, causes an effect there, and then leaves the brain, but the effect (eg, volume loss) continues over time as a function of cumulative lead dose.

Multiple linear regression was used to evaluate associations of predictor variables with change in brain volumes, using both ROI-based and voxel-wise approaches and change in CHS scores. All regression models were adjusted for baseline age, duration of time between MRIs, apolipoprotein E genotype, peak tibia lead (in analysis with former lead workers only), control status (ie, former lead worker vs control, in analysis with both only), baseline ROI volume, height (cm), and education.[14] Model diagnostics were used to evaluate influence and normality for the ROI-based analysis.

ROI-Based Approach

To be consistent with the results of our previous published reports, we modeled change in 20 previously selected ROI volumes.[8] For bilateral structures, the volume represented the sum of right and left structures to minimize multiplicity concerns, but analyses were also performed separately for change in left- and right-sided ROI volumes (data not reported). Because we did not formally adjust for multiple comparisons in the ROI analysis, we acknowledge that a P-value < 0.05 does not necessarily imply statistical significance.

TABLE 3. Summary Statistics for Change in Selected Region of Interest Volume Measures for 353* Former Lead Workers and Population Controls Without a History of Occupational Exposure to Lead

	Delta ROI† (cm^3)		Delta ROI/TBV1 (%)	
ROI‡	Mean (SD)	MIN, MED, MAX	Mean (SD)	MIN, MED, MAX
TBV	−29.87 (24.34)	−98.08, −31.62, 72.19	−2.55 (2.09)	−8.48, −2.69, 6.88
VENTRICLES	−0.53 (2.75)	−8.71, −0.73, 21.1	−0.043 (0.24)	−0.72, −0.06, 1.74
TOTAL GM	−24.43 (17.45)	−77.35, −24.74, 47.50	−2.09 (1.50)	−6.67, −2.19, 4.53
FRONT GM	−2.95 (5.18)	−23.99, −2.75, 14.49	−0.25 (0.44)	−2.09, −0.24, 1.46
OCCIP GM	−3.30 (1.82)	−8.42, −3.37, 2.75	−0.28 (0.15)	−0.75, −0.29, 0.26
PARI GM	−3.81 (2.67)	−13.36, −3.83, 7.40	−0.33 (0.23)	−1.16, −0.33, 0.71
TEMP GM	−2.69 (4.42)	−14.42, −2.76, 15.55	−0.23 (0.38)	−1.25, −0.22, 1.48
TOTAL WM	−5.44 (13.48)	−48.33, −6.41, 42.80	−0.46 (1.16)	−4.23, −0.55, 3.49
FRONT WM	−4.37 (5.50)	−27.72, −4.21, 19.00	−0.37 (0.46)	−2.42, −0.36, 1.55
OCCIP WM	0.56 (1.67)	−5.23, 0.51, 5.93	0.05 (0.14)	−0.41, 0.05, 0.52
PARI WM	−1.02 (3.03)	−10.76, −0.88, 12.39	−0.09 (0.26)	−0.94, −0.08, 1.01
TEMP WM	−2.40 (3.18)	−12.80, −2.35, 6.61	−0.20 (0.27)	−1.12, −0.20, 0.61
ERC	−0.30 (0.24)	−1.20, −0.29, 0.43	−0.03 (0.02)	−0.10, −0.03, 0.04
AMYG	−0.25 (0.21)	−1.02, −0.25, 0.49	−0.02 (0.02)	−0.09, −0.02, 0.05
HIPPO	−0.48 (0.39)	−1.89, −0.47, 0.87	−0.04 (0.03)	−0.16, −0.04, 0.09
CEREB	−3.42 (4.40)	−14.73, −4.27, 18.74	−0.30 (0.37)	−1.52, −0.36, 1.41
MEDIAL	−4.53 (3.03)	−13.63, −4.51, 7.89	−0.39 (0.25)	−1.19, −0.39, 0.75
INSULA	−1.26 (0.76)	−3.90, −1.17, 1.08	−0.11 (0.06)	−0.34, −0.10, 0.10
CINGULATE	−0.40 (1.26)	−5.76, −0.43, 4.69	−0.03 (0.11)	−0.50, −0.04, 0.45
CORP CALL	−0.96 (0.48)	−2.81, −0.88, 0.47	−0.08 (0.04)	−0.25, −0.08, 0.05
INT CAPS	−0.28 (0.44)	−2.31, −0.25, 1.09	−0.02 (0.04)	−0.20, −0.02, 0.09

*Of the 362 persons with two MRIs, eight former lead workers and one control had first MRIs that were of insufficient quality for analysis.
†Delta ROI = volume at second MRI minus volume at first MRI; all volumes combine bilateral structures.
‡TBV, total brain volume (TBV1, TBV at first MRI); GM, gray matter; FRONT, frontal; OCCIP, occipital; PARI, parietal; TEMP, temporal; WM, white matter; ERC, entorhinal cortex; AMYG, amygdala; HIPPO, hippocampus; CEREB, cerebellum; MEDIAL, medial structures (bilateral amygdala, cuneus, entorhinal cortex, hippocampal formation, lingual gyrus, medial front-orbital gyrus, medial frontal gyrus, medial occipito-temporal gyrus, parahippocampal gyrus, perirhinal cortex, precuneus, and uncus); CORP CALL, corpus callosum; INT CAPS, internal capsule.

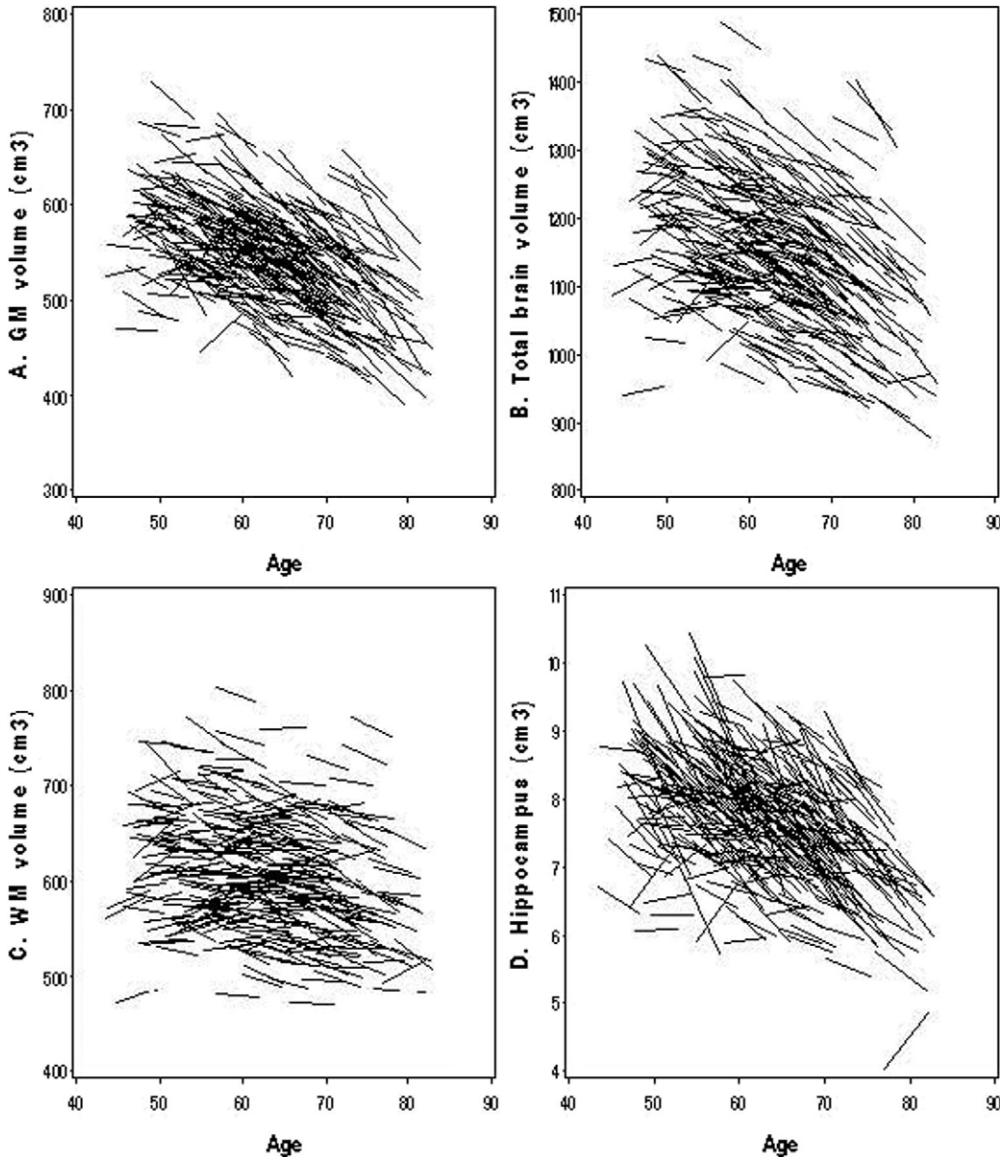

FIGURE 1. "Spaghetti plots" for relations of age with change (cm^3) in four ROI volumes (panel A, total gray matter; panel B, total brain; panel C, total white matter; and panel D, bilateral hippocampus), including former lead workers and controls. Each line represents an individual's change in age and change in ROI volume across the two MRIs.

Voxel-Wise Approach

Change in voxel volumes was modeled controlling for the aforementioned covariates using multivariate permutation testing in the R statistical programming language (www.cran.r-project.org). The SPM5 package (Statistical Parametric Software, Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, University College London, 2003) was used to perform smoothing using a 3D isotropic Gaussian filter and MRIcro[24] to display results. Statistical significance was evaluated using a permutation approach that controlled for confounding variables. The maximum cluster size and cluster peak above the threshold were used to define a conservative permutation distribution on cluster sizes and peaks that, when compared with the observed cluster sizes and peaks, controls for multiple comparisons.

White Matter Lesions

Linear regression was used to model change in CHS white matter lesion grade scores.

RESULTS

Descriptive Summary of Study Subjects

Compared with those with no or one MRI, subjects with two MRIs were slightly younger, had a shorter time since last occupational exposure to lead, and lower peak tibia lead levels (Table 1). The mean (SD) duration from the first MRI to the second was 5.0 (0.4) years (range, 3.6 to 6.1 years). The current age of the 317 former lead workers and 45 controls was 64.1 (7.6) and 71.9 (6.0) years, respectively ($P < 0.001$; Table 2). Among all cohort members, controls had higher current tibia lead levels than did former workers (mean of 19.0 vs 14.8 μg/g; Table 1), likely due to a cohort effect associated with the higher average age of controls.

Descriptive Summary of Change in ROI Volumes

There was no evidence that declines in volumes differed between former lead workers and controls (data not shown); hence, results are presented for all subjects combined. On an average, total brain volume declined almost 30 cm^3 during a 5-year period (Table

3 and Fig. 1), with a more substantial decline in gray (24.4 cm^3) compared with white (5.4 cm^3) matter. All ROIs evidenced decline except for occipital white matter. The mean (SD) percent gray matter at the first MRI was 47.9% (1.8%) and at the second MRI was 47.0% (1.9%). When change is expressed as a percent of the baseline total brain volume, the greatest decline was observed for total brain (2.55%), followed by total gray matter (2.09%), total white matter (0.46%), and medial structures (0.39%).

Predictors of Change in ROI Volumes

Among former lead workers, peak tibia lead was not associated with change in ROI volumes in adjusted models (Table 4). The remaining predictors were evaluated in all subjects to maximize power, given the lack of association for tibia lead and lack of differences between former lead workers and controls. As baseline age increased, ROI volumes declined (Table 4), with the expected exception of ventricle volume which increased in relation to baseline age. A larger ROI volume at baseline was associated with a greater decline in volume, a finding expected from regression toward the mean (data not shown). Larger durations between MRIs were associated with larger declines in gray matter volumes, larger increases in white matter volumes, and larger increases in ventricle volume (Table 4).

Predictors of Change in Voxel Volumes

In a parallel analysis, results were substantively similar using a voxel-wise approach. For example, suprathreshold clusters for the association of lead with change in volume were well within the range expected by chance. In contrast, the adjusted association between baseline age and change in voxel volumes identified large suprathreshold clusters, whose sizes were well above the distribution of the maximum cluster size under the null hypothesis (Fig. 2).

Predictors of Change in CHS White Matter Lesion Grade Score

For the change in CHS white matter lesion grade score (CHS2 minus CHS1), 6 persons (1.7%) improved by one category, 87 persons (24.0%) were unchanged, 134 persons (36.9%) worsened one category, 100 persons (27.6%) worsened by two categories, 24 persons (6.6%) by three categories, and 12 (3.3%) by four or five categories. Neither peak tibia lead nor control status were associated with change in CHS sores. Baseline age and increasing duration between MRIs were associated with increases in CHS scores (beta = 0.055, $P < 0.001$ and beta = 0.286, $P = 0.05$, respectively).

DISCUSSION

In this cohort of 45- to 75-year-old men with past occupational exposure to organic and inorganic lead and population controls, we had previously observed that peak tibia lead concentration (an estimate of past cumulative lead dose) was associated with worse neurobehavioral test scores at cross section,[5] longitudinal decline in cognitive function,[6] the prevalence and severity of white matter lesions, and with decreased volumes in both larger (eg, total brain, lobar gray and white matter) and smaller (eg, cingulate

TABLE 4. Linear Regression[a] Results for Delta ROI Models for Former Lead Workers and Controls ($N = 352$), Adjusting for Confounding Variables

	Beta (SE)		
ROI[b]	**Baseline Age**	**Duration Between MRIs Beta (SE)**	**Peak Tibia Lead**
TBV	−1.771 (0.154)***	−2.533 (3.002)	0.1496 (0.0865)
VENTRICLES	0.102 (0.021)***	1.230 (0.387)***	−0.0001 (0.0111)
TOTAL GM	−1.072 (0.119)***	−7.162 (2.260)***	0.0906 (0.0646)
FRONT GM	−0.350 (0.035)***	−1.473 (0.658)**	0.0217 (0.0186)
OCCIP GM	−0.072 (0.012)***	−0.866 (0.245)***	0.0184 (0.0071)**
PARI GM	−0.163 (0.018)***	−0.523 (0.345)	0.0137 (0.0102)
TEMP GM	−0.252 (0.029)***	−1.528 (0.561)***	0.0245 (0.0161)
TOTAL WM	−0.758 (0.089)***	4.904 (1.779)***	0.0544 (0.0505)
FRONT WM	−0.185 (0.039)***	1.201 (0.763)	0.0224 (0.0220)
OCCIP WM	−0.064 (0.012)***	0.618 (0.232)***	−0.0047 (0.0065)
PARI WM	−0.097 (0.021)***	1.014 (0.429)**	0.0174 (0.0124)
TEMP WM	−0.107 (0.022)***	1.436 (0.446)***	0.0104 (0.0128)
ERC	−0.005 (0.001)***	−0.0004 (0.028)	0.0013 (0.0008)
AMYG	−0.008 (0.001)***	0.054 (0.028)*	0.0001 (0.0008)
HIPPO	−0.015 (0.003)***	0.090 (0.050)*	−0.0002 (0.0015)
CEREB	−0.213 (0.027)***	−0.749 (0.540)	−0.0125 (0.0157)
MEDIAL	−0.188 (0.020)***	−0.217 (0.380)	0.0077 (0.0110)
INSULA	−0.029 (0.005)***	0.084 (0.833)	0.0044 (0.0029)
CINGULATE	−0.081 (0.007)***	0.001 (0.142)	0.0058 (0.0042)
CORP CALL	0.005 (0.003)	0.053 (0.065)	0.0002 (0.0019)
INT CAPS	−0.010 (0.003)***	0.026 (0.060)	0.0001 (0.0018)

*$0.05 < P < 0.10$; **$0.01 < P < 0.05$; ***$P < 0.01$.

[a]Regressions also included *APOE* genotype (2–3, 2–4, and 3–4 plus 4–4, each compared with 3–3 as reference group), height, baseline ROI, control status, duration between MRIs, and education. The model with peak tibia lead was in former lead workers only.

[b]TBV, total brain volume (TBV1, TBV at first MRI); GM, gray matter; FRONT, frontal; OCCIP, occipital; PARI, parietal; TEMP, temporal; WM, white matter; ERC, entorhinal cortex; AMYG, amygdala; HIPPO, hippocampus; CEREB, cerebellum; MEDIAL, medial structures (bilateral amygdala, cuneus, entorhinal cortex, hippocampal formation, lingual gyrus, medial front-orbital gyrus, medial frontal gyrus, medial occipito-temporal gyrus, parahippocampal gyrus, perirhinal cortex, precuneus, and uncus); CORP CALL, corpus callosum; INT CAPS, internal capsule.

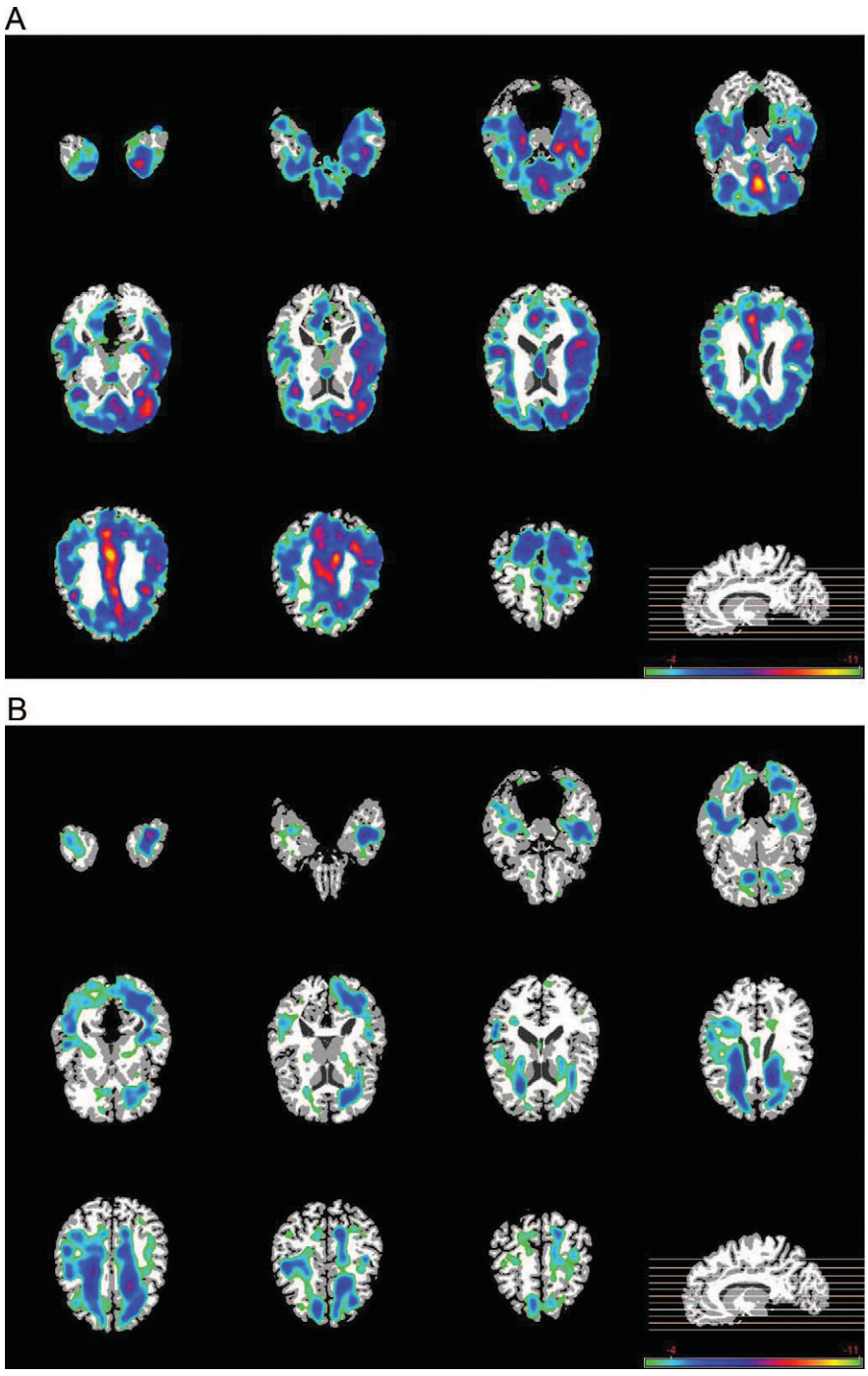

FIGURE 2. Transverse template brain slices with *t* statistic maps of the adjusted association between age and change in brain volumes on a voxel-wise basis. Location of slice is identified by figure in lower right corner. The figure displays *t* statistics <-3.1 with colors defined by key in lower right corner. Panel A is for gray matter, and panel B is for white matter. For gray matter, the maps identified one large cluster that exhibited both maximum cluster size and peak value significance after controlling for multiplicity (*P*-values < 0.05). For white matter, there were 28 clusters that satisfied these two criteria.

gyrus, insula, corpus callosum) ROIs,[8] almost two decades after occupational lead exposure had ended. Because tibia lead was not associated with change in brain volumes over time using both ROI- and voxel-based methods, the current analysis suggests that the influence of lead on brain structure is persistent, but the results do not support progressive changes during the 5 years as measured by volumes in two MRIs. Our previous reports of progressive cognitive decline associated with past cumulative lead dose, which we termed "accelerated aging," may be explained by a persistent lead-associated structural lesion combined with the effect of other risk factors associated with aging.[1,6,7] That is, cognitive decline in subjects without occupational lead exposure is age dependent but is more rapid when aging is combined with such exposure, even after exposure ceases. However, it should be noted that a portion of what has been previously termed age-related cognitive decline may be due, at least in part, to ubiquitous neurotoxicants such as lead or mercury.[1,25,26]

More specifically, in the previous cross-sectional analysis,[8] the association of tibia lead with brain volumes and white matter lesions was evidence of a persistent influence of lead on brain structure. In that analysis, the studied former workers had a mean (SD) lead exposure duration of 8.7 (9.8) years and a mean (SD)

duration since last occupational exposure to lead of 18.0 (11.0) years. This implies that the lower brain volumes and increased prevalence and severity of white matter lesions associated with tibia lead levels in the cross-sectional analysis could reflect changes that had occurred over as much as the previous 26.7 years, since the beginning of occupational lead exposure. In the current analysis, we did not observe additional longitudinal change associated with cumulative lead dose during the next 5 years. Thus, we conclude that cumulative lead dose was associated with persistent but not likely progressive structural changes in the brain. These findings are also not inconsistent with our previous conclusion that at least part of the influence of cumulative lead dose on cognitive function is mediated through volume loss,[11] for the same reasoning as above regarding the differing time periods of opportunity for change associated with lead dose to occur in the cross-sectional and longitudinal analyses.

Our data on the magnitude of changes in MRI volumes associated with age and aging are similar to those previously reported with some notable differences.[27–33] Our whole brain atrophy rate of ~0.5% per year is similar to values reported in some previous studies[27,29,30] but not others.[34,35] Resnick et al[27] reported slightly larger losses in white matter than gray matter in an older population of 50 men and 42 women; white matter losses were widespread, whereas gray matter losses were more localized.[36] In a study of 362 volunteers ranging in age from 18 to 93 years, whole-brain volume adjusted for head size declined by 0.22% per year between 20 and 80 years, then more rapidly after that.[28] In an earlier report of 370 adults ranging from 18 to 97 years of age, the rate of decline in old nondemented subjects was 0.45% per year,[37] with the observation that gray matter volume loss began at age 20 and continued to very old age. For the latter study, the white matter loss seems to begin in the fifth or sixth decade, a finding consistent with our estimates of the relative amount of gray and white matter losses.

An important consideration is whether selection bias could account for the results we have reported. After the first MRI, we determined that average cognitive function did not differ by first MRI status and the relations of tibia lead with neurobehavioral test scores did not differ in those with and without MRIs.[8] After the first MRI, we concluded there was unlikely to be meaningful selection bias among those who completed the first MRI that could influence study results.[8] Former lead workers with two MRIs had lower tibia lead levels and were younger than those with only one (Table 1). We believe these differences are likely to mask, rather than spuriously create, associations.

A fundamental methodological challenge in longitudinal MRI studies is posed by changes in scanner hardware and software between scans. Initial analysis showed that applying standard 3D segmentation methods independently to each scan was insufficient and led to low longitudinal stability of the volumetric measurements. We therefore used an advanced four-dimensional segmentation and atlas registration technique, which has been developed and validated specifically for longitudinal studies.[38] A potential pitfall of this approach is that it can over smooth and therefore underestimate longitudinal brain changes, if the parameters that control temporal smoothness are not properly set. However, previous validation studies of this approach carefully determined the appropriate parameter range.

In conclusion, in this cohort of former lead workers, cumulative lead dose was associated with persistent effects on brain volume, but recent changes in brain volume during 5 additional years were not associated with tibia lead. Advancing age is associated with annual declines in brain volumes of ~0.5% per year, primarily in gray matter in this age range.

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