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Abstract—*Objective:* To determine whether cumulative lead dose in former organolead workers was associated with MRI measures of white matter lesions (WML) and global and structure-specific brain volumes. *Methods:* MRIs, tibia lead, and other measures were obtained from 532 former organolead workers with a mean age of 56 years and a mean of 18 years since last occupational exposure to lead. Cumulative lead dose was measured by tibia lead, obtained by X-ray fluorescence, and expressed as μg lead per gram of bone mineral (μg Pb/g). WML were evaluated using the Cardiovascular Health Study grading scale. A total of 21 global and specific brain regions were evaluated. *Results:* A total of 36% of individuals had WML grade of 1 to 7 (0 to 9 scale). Increasing peak tibia lead was associated with increasing WML grade ($p = 0.004$). The adjusted OR for a 1 μg Pb/g increase in tibia lead was 1.042 (95% CI = 1.021, 1.063) for a CHS grade of 5+ (≥5 vs <5). In linear regression, the coefficient for tibia lead was negative for associations with all structures. Higher tibia lead was significantly related to smaller total brain volume, frontal and total gray matter volume, and parietal white matter volume. Of nine smaller specific regions of interest, higher tibia lead was associated with smaller volumes for the cingulate gyrus and insula. *Conclusions:* These data suggest that cumulative lead dose is associated with persistent brain lesions, and may explain previous findings of a progressive decline in cognitive function.

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We previously reported on the relation between past occupational exposure to organic (i.e., tetraethyl and tetramethyl lead) and inorganic lead and decline in cognitive function. Among those initially enrolled in the study, there was an average of 16 years since last occupational lead exposure.[1,2] Past cumulative absorption of lead, estimated from peak tibia leads, was associated with a decline in neurobehavioral test scores, with pronounced longitudinal declines in verbal memory and learning, visual memory, and executive function.[1,2] Subsequent analysis supported our hypothesis that cumulative lead dose was associated with progressive declines in cognitive function long after lead levels had declined in brain and blood.[3] Based on these findings, we hypothesized that the decline in function associated with tibia lead could have been mediated by brain lesions that were at least persistent. This hypothesis motivated the current study in which we obtained structural MRIs of the brain. Because tibia lead was associated with declines in a broad range of cognitive domains (i.e., verbal memory and learning, visual memory, manual dexterity, and executive abilities), we predicted that it would be associated with decline in volumes in several structures, ranging from large (e.g., total brain, frontal lobe, parietal lobe) to small (e.g., specific structures involved in learning and memory such as limbic and perilimbic volumes).

The hypothesis that lead may cause persistent brain lesions is consistent with animal evidence suggesting that lead can cause cell death or changes to cellular architecture[4-12] including the formation of neurofibrillary tangles.[10,12] Moreover, glial acidic fibrillary protein, a cell-specific cytoskeletal intermediate filament protein that is an indicator of cell damage or death, increases in the hippocampus of animals dosed with lead.[4,7,11] Herein, we describe results of a follow-up study in this same cohort to evaluate whether tibia lead levels were associated with MRI measures of white matter lesions and total and region specific brain volumes.

Editorial, see page 1464

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Table 1 *Profile of data collected on former organolead workers during phases I and II*

MRI completed in phase II	Tibia lead measured	Recruited during		
		Phase I	Phase II	Total
No	No	118	9	127
No	Yes	223	40	263
Yes	No	5	52	57
Yes	Yes	357	175	532*
Total		703	276	979

* These subjects are the focus of the analysis presented herein.

Methods. Data were derived from individuals recruited during two study phases between 1994 and 2003 (table 1). In phase I (1994 to 1997) former employees of a chemical manufacturing plant in the eastern United States were identified and recruited. Annual measures of neurobehavioral and peripheral nervous system function were obtained. Tibia lead concentration was measured by ^{109}Cd-induced X-ray fluorescence (XRF) during the third year of phase I. In phase II (2001 to 2003), we continued to study the cohort, enrolled additional study participants, and collected tibia lead and MRI data. The detailed methods for phase I are described elsewhere[1,2] and summarized herein, in brief, along with specific details of the phase II study. The study was 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.

Individuals recruited for this study worked in the organolead area of a chemical plant, involved in the manufacture of tetraethyl lead from 1923 to 1991 and tetramethyl lead from 1960 to 1983,[13] but were not occupationally exposed to lead at the time of study enrollment. All study participants were previously employed in the facility on or after January 1, 1950, were men, and were between the ages of 40 and 70 years in 1995. In phase I, a total of 703 former lead workers were enrolled and completed one to four visits. In phase II, another 276 former lead workers were enrolled and completed one or two visits. In addition, during phase II, MRIs were completed on 589 of the 979 former lead workers (see table 1). Tibia lead was measured on 532 of the 589 individuals who completed MRI acquisition. Analysis was limited to these 532 individuals.

Data collection. After obtaining consent, blood pressure, height, and weight, questionnaire interview, and neurobehavioral and psychological testing data were obtained[1] as well as two 10-mL blood specimens by venipuncture. The remaining description is confined to measures specifically used for the analysis presented herein.

During each interview, data were collected on use of cigarettes and cigars, including age started smoking, average number of cigarettes smoked per day, ever quit for a week or more, longest duration of time stopped smoking, and age stopped smoking. Similar data were collected on alcohol consumption and also included types of beverage consumed. We examined a variety of approaches to summarizing use of tobacco and alcohol, including cumulative use combined with current use status. We opted for current use status (i.e., never, current, or past use) because it was the simplest approach and explained as much variance in MRI outcomes as alternative approaches.

Elevated blood pressure and hypertension are each associated with risk of white matter lesions and total brain volume,[14-19] as well as lead exposure, and thus were initially considered as potential confounding variables. During each visit a question was asked about history of hypertension. Affirmative responses were followed by questions about current antihypertensive medications. Systolic and diastolic blood pressures were measured during each study visit as previously described.[20] For the present analysis, we used the available blood pressure measures closest in time to when MRI data were obtained. Individuals were defined as hypertensive if their systolic blood pressure was greater than 140, their diastolic blood pressure was greater than 90, or they were using medications to control blood pressure.

Current tibia lead was measured at the left mid-tibia shaft for 30 minutes by ^{109}Cd-induced K-shell X-ray fluorescence (XRF).[21,22] Of the 532 former lead workers on whom MRI and tibia lead measurements were completed (see table 1), tibia lead was obtained from 357 subjects in phase I and 175 subjects in phase II. A limitation of current tibia lead for estimation of cumulative occupational lead dose in former workers exposed to lead is that it declines in relation to time since last work in lead-exposed jobs. As such, two individuals with the same cumulative occupational dose will differ in their current tibia lead level if they differ in their time since last occupational exposure to lead. To account for the large interindividual differences in time since last occupational lead exposure, years since last exposure was used to estimate tibia lead levels at the termination of lead exposure, termed peak tibia lead (PTL). Current tibia lead levels were extrapolated back using a clearance half-time of lead in tibia of 27 years,[23] assuming first-order (mono-exponential) clearance from tibia.[1,2] PTL is expressed in units of μg lead per gram of bone mineral (μg Pb/g). In previous work, we showed that PTL was the best predictor of decline in cognitive function in this cohort.[3]

Apolipoprotein E genotyping was completed using two different methods in phase I and phase II. The method of Hixson and Vernier[24] was used during phase I. In phase II, genotyping was performed using the Flexigene DNA Kit (Qiagen, Valencia, CA). The method was previously validated in a subset of individuals from another study using published PCR conditions[25] and described elsewhere.[26]

MRI measures. In this section we describe the methods used to acquire MRIs, to obtain white matter lesion severity scores, and to quantify volumes of brain structures.

All subjects were imaged at the same location and on the same General Electric 1.5 T Signa model. A set of 1.5 mm T1-weighted images through the entire brain were acquired, using contiguous coronal MR images and a spoiled gradient recalled acquisition in steady state sequence (echo time [TE] = 5 msec and repetition time [TR] = 35 msec). Field of view was 24 cm and the matrix size was 256 × 256. Proton density and T2-weighted axial dual-echo images were also acquired parallel to the anterior-posterior commissure line, using an interleaved technique with no gap to provide maximal data for subsequent segmentation and ratings of white matter disease.

White matter lesions were graded based on review of the proton density and T2-weighted scans using the Cardiovascular Health Study 10-point (0 to 9) scale.[17,27] Predefined visual standards of nine reference cases were used to grade the periventricular and subcortical white matter signal abnormality (table 2, footnote) on spin density weighted axial images that successively increased from no (grade 0) or barely detectable change (grade 1) to almost all white matter involved (grade 9). Portions of these atlases have been previously published.[28] The Cardiovascular Health Study scale has high inter-reader and intrareader reliability.[29] Volumetric analytic validation of the visual scale corresponded to a rank increase in white matter T2W hyperintensity normalized for cerebral parenchymal volume.[30,31]

Cardiovascular Health Study grading was completed in full DICOM format (Merge Efilm, Milwaukee, WI). All MRIs were read by a board-certified experienced neuroradiologist also trained to meet specified reader reproducibility criteria for the Cardiovascular Health Study. The reviewer was blinded to PTL level and lead exposures status. The sagittal T1-weighted, axial proton density and T2-weighted, and coronal spoiled gradient echo scans were reviewed in the same setting.

MR images were segmented into gray matter (GM), white matter, and CSF fluid, as described by Goldszal et al.[32] Regional volumetric analysis was then performed via computerized template matching techniques previously reported and validated.[33] In particular, a digital atlas bearing anatomic definitions of several brain regions was used as reference. The atlas included all major lobar subdivisions as well as a number of smaller regions (see Results). A computerized image analysis algorithm based on pattern matching was then used to warp this reference atlas to each participant's MRI, thereby transferring the anatomic definitions onto the MRI. As a result, volumetric measurements of the regions of interest (ROI) defined in the atlas were obtained from each participant. Gray matter and white matter volumetric measurements were examined individually and in sum.

Statistical analysis. The primary purpose of the analysis was to determine if PTL was associated with white matter lesions or

Table 2 *Logistic regression estimate of the unadjusted and adjusted OR for peak tibia lead (PTL) and relation to white matter grade* using five different cut-points, 532 former organolead workers, 2001–2003*

White matter (WM) grade cutpoint†	No. cases	Unadjusted OR (95% CI)‡	Adjusted§OR† (95% CI)‡	*p* Value	OR for interquartile¶ (95% CI)
WM grade ≥ 1 vs < 1	180	1.025 (1.014–1.035)	1.006 (0.994–1.018)	0.35	1.10 (0.89–1.41)
WM grade ≥ 2 vs < 2	120	1.033 (1.021–1.044)	1.017 (1.004–1.031)	0.01	1.38 (1.09–1.79)
WM grade ≥ 3 vs < 3	69	1.037 (1.024–1.050)	1.019 (1.004–1.033)	0.01	1.41 (1.08–1.88)
WM grade ≥ 4 vs < 4	45	1.049 (1.034–1.064)	1.029 (1.012–1.046)	<0.001	1.74 (1.26–2.39)
WM grade ≥ 5 vs < 5	25	1.060 (1.042–1.079)	1.042 (1.021–1.063)	<0.001	2.21 (1.49–3.28)

* Cardiovascular Health Study grades are defined as follows (grade in parenthesis): no WM abnormalities (0); discontinuous periventricular (PV) rim or minimal "dot" of subcortical (SC) disease (1); thin continuous PV rim or few patches of SC disease (2); thicker continuous PV rim with scattered patches of SC disease (3); thicker shaggier PV rim and mild SC disease (4); mild PV confluence surrounding the frontal and occipital horns (5); moderate PV confluence surrounding the frontal and occipital horns (6); PV confluence with moderate involvement of the centrum semiovale (7); PV confluence involving most of the centrum semiovale (8); all supratentorial WM involved (excluding the corpus) (9).

† Defined as the individuals who had a white matter grade at or above the cut-point.

‡ The OR for a 1 μg/g increase in PTL.

§ Adjusted for age (linear term was significant), education, ApoE-ε4 status (i.e., at least one ε4 allele), and smoking status (previous, current, never smoker).

¶ The OR associated with an increase in PTL from the 25th to the 75th percentile, in this case, and increase from 12.3 to 31.6 μg/g Pb.

with volume of specific brain regions, selected a priori. In these analyses, one individual was excluded from all analysis based on MRI evidence of hydrocephalus.

We tested whether the OR for the association of PTL and white matter grade increased with increasing white matter grade. Logistic regression was used to model the relation between white matter grade and PTL, adjusting for potential confounders, using five separate regression models. For each model, PTL was defined as a continuous variable and white matter grade was defined as a binary variable. In sequence, an increasing white matter grade value was used to define the binary cut-point for each of five logistic models (i.e., white matter grade ≥ 1 vs < 1; white matter grade ≥ 2 vs < 2; white matter grade ≥ 3 vs < 3; white matter grade ≥ 4 vs < 4; white matter grade ≥ 5 vs < 5), to examine sensitivity of results to the prevalence and severity of lesions. In addition, we used a polytomous logistic model to test for overall trend of increasing OR in relation to increasing white matter grade by defining white matter grade as an ordinal outcome (i.e., 0, 1, 2, 3, 4, 5+). In developing the final set of models we considered the following as potential confounders: age, systolic and diastolic blood pressure, smoking history, ApoE genotype, education, alcohol consumption, depression status, and race. Independent covariates were retained in the model if they had at least a borderline significant ($p < 0.10$) relation to one or more white matter binary outcomes or the sign for the coefficients was in the same direction more than 75% of the time (i.e., either negative or positive) and supported by prior evidence of an association with white matter grade. The final model included peak tibia lead, age at which the MRI was obtained (linear term was significant, quadratic term was not), education (i.e., less than high school, high school diploma, some college, college degree or more), ApoE-ε4 status (i.e., at least one ε4 allele), and tobacco use status (i.e., current or past user vs nonuser).

Eighteen of the MRIs were not suitable for volumetric analysis due to image quality. No significant or meaningful differences were found on any covariates measured between these 18 individuals and the remaining 514 individuals with suitable MRIs. A total of 514 MRIs were segmented into gray matter, white matter, and CSF,[32] then regional volumetric analysis was performed via computerized template matching techniques.[33] A total of 91 possible brain regions of interest (ROIs) were generated from a digital brain atlas. For analysis, we focused on total brain volume, total white and gray matter, lobar white and gray matter, and a select set of nine more specific ROIs (table 3, "other structures"). All volumes were expressed as the sum of the same ROI from the right and left sides, where relevant.

Multiple linear regression[34] was used to evaluate associations of PTL with each ROI-specific volumetric measure. Each ROI-specific outcome was modeled separately. Some of the dependent variables are not independent of each other because some are nested within other structures (e.g., total brain volume, total lobar volumes, lobar gray or white matter volumes). This approach was used to determine if there were global and specific effects of lead. In linear regression, unadjusted and adjusted coefficients were estimated for the association between PTL and volumetric measures. Models were first evaluated with only PTL and age (i.e., the age on the date the MRI was acquired). Previous studies indicated that age was strongly related to volume. We examined linear and quadratic terms for age, but only the linear term was significantly associated with volumetric measures. Other suspected confounders considered in the analysis included systolic and diastolic blood pressure, smoking history, ApoE genotype, education, alcohol consumption, depression status, and race. Finally, we also assumed height would be associated with ROIs, as it is directly associated with the size of body structures. In evaluating covariates for each linear regression model, we used a backward elimination procedure and the aforementioned criteria for logistic regression. Age, education, ApoE-ε4 status, and height were retained in the final model. The coefficients for systolic and diastolic blood pressure and ROI volume were often negative, indicating that lower brain volume is associated with higher blood pressure. However, previous analysis[20] of a direct relation between PTL and blood pressure level indicated that blood pressure was more likely to be in the causal pathway between PTL and neurodegenerative changes than to act as a confounding variable. We therefore did not include systolic or diastolic blood pressure in the final ROI models. Ultimately, whether or not blood pressure was included in the regression models did not influence interpretation of the relation between PTL and ROI volume.

All linear regression models were evaluated for violations of the assumptions of linear regression and for influence of outliers in PTL and volume measures.

Results. The 532 former workers in this analysis were, on average, 56.1 years of age and had exposure duration of 8.7 years (table 4). The average time interval between last occupational exposure to lead and when the MRI was obtained was 18 years. Individuals were predominantly white and more than 90% completed high school; approximately 35% had some college. Compared to nonparticipants (i.e., the 447 participants without an MRI) the MRI group did not differ by age, race/ethnicity, tobacco use, or blood pressure, but did have a greater mean exposure duration ($p < 0.02$) and proportionately fewer individuals with less than a high school education ($p < 0.02$) and who reported previous alcohol consumption ($p < 0.03$). The mean current tibia lead for the MRI group was 14.5 μg Pb/g compared to 15.7 μg Pb/g among nonparticipants. The

Table 3 *Global and region specific volumetric measures (mL) for 514 former organolead workers and linear regression coefficients (unadjusted and adjusted) for the relation between peak tibia lead (PTL) and volumes, former organolead manufacturing workers, 2001–2003*

						Peak tibia lead regression coefficients					
						Unadjusted			Adjusted†		
No.	Structure*	Mean (mL)	SD (mL)	Min (mL)	Max (mL)	β (mL)	SE	*p*	β (mL)	SE	*p*
Total											
1	Total brain volume‡	1,150.49	105.84	733.56	1,487.99	–1.141	0.249	<0.001	–0.609	0.273	0.03
White matter											
2	Frontal	203.42	22.75	142.27	277.14	–0.195	0.054	0.0003	–0.076	0.058	0.19
3	Parietal	104.75	12.47	73.29	146.62	–0.102	0.030	0.0006	–0.070	0.032	0.03
4	Temporal	109.83	13.55	68.52	152.75	–0.122	0.032	0.0002	–0.067	0.035	0.06
5	Occipital	46.11	7.16	27.83	70.68	–0.029	0.017	0.09	–0.011	0.019	0.55
6	Total‡	562.00	58.99	389.48	748.22	–0.531	0.140	0.0002	–0.282	0.152	0.06
Gray matter											
7	Frontal‡	149.53	16.21	107.68	201.05	–0.214	0.038	<0.001	–0.094	0.041	0.02
8	Parietal	87.18	9.83	50.58	121.97	–0.099	0.023	<0.001	–0.046	0.025	0.07
9	Temporal	109.39	14.01	48.14	147.03	–0.109	0.033	0.001	–0.059	0.037	0.11
10	Occipital	53.24	6.42	27.74	77.07	–0.047	0.015	0.002	–0.022	0.017	0.19
11	Total‡	588.49	59.97	314.35	762.85	–0.609	0.141	<0.001	–0.326	0.157	0.04
Other structures											
12	Medial§	89.60	10.69	39.84	124.29	–0.087	0.025	0.0007	–0.039	0.028	0.17
13	Cingulate	22.52	3.84	6.48	34.74	–0.055	0.009	<0.001	–0.025	0.010	0.008
14	Insula	13.88	1.90	6.96	21.64	–0.018	0.004	<0.001	–0.012	0.005	0.02
15	Corpus callosum	11.90	1.65	6.58	18.39	–0.006	0.004	NS	–0.008	0.004	0.07
16	Internal capsule	7.66	0.87	4.81	10.84	–0.007	0.002	0.0009	–0.002	0.002	0.40
17	Amygdala	2.42	0.64	0.48	3.96	–0.001	0.002	NS	–0.002	0.002	0.23
18	Hippocampus	6.92	1.55	0.82	10.18	–0.007	0.004	0.07	–0.005	0.004	0.24
19	Entorhinal cortex	3.09	0.71	1.00	5.63	–0.001	0.002	NS	–0.001	0.002	0.56
20	Cerebellum	113.53	21.20	43.82	160.43	–0.036	0.051	NS	–0.068	0.057	0.24

* Bilateral volume for all structures.

† Adjusted for age (linear term was significant), education, ApoE-ε4 status (i.e., at least one ε4 allele), and height.

‡ One subject with apparent hydrocephalus was removed from this analysis due to extreme values.

§ Medial structures encompasses 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. These structures mediate the limbic system functions.

mean PTL was 23.9 μg Pb/g in the MRI group, significantly lower ($p < 0.02$) than the 27.1 μg Pb/g among nonparticipants.

White matter grade. Overall, 34% of individuals (see table 2) had a Cardiovascular Health Study white matter grade of 1 or greater; of these, 77% were less than or equal to 3. In logistic regression, the strength of the association between PTL and white matter grade increased as a higher grade was used to define the outcome (see table 2). Significant associations were observed when a white matter grade of 2 or greater was used, indicating that PTL was associated with both the prevalence and severity of white matter lesions. Specifically, the adjusted OR for a 1 μg/g increase in PTL (modeled as a continuous variable) increased as the cut-point for the binary outcome increased from greater than or equal to one (OR = 1.006, $p > 0.05$) to greater than or equal to five (OR = 1.042, $p < 0.001$). For the last model tested (i.e., white matter grade ≥ 5 vs < 5), the adjusted OR associated with an increase of PTL from 12.3 to 31.6 μg Pb/g (the interquartile range for PTL) was 2.21 (see table 2). In polytomous logistic regression (i.e., white matter grade defined as an ordinal variable), increasing PTL was associated with increasing severity of white matter lesions ($p = 0.004$).

Volumetric analysis. The mean (SD) values for the 20 volumetric measures ranged from 2.4 (0.6) mL for bilateral amygdala to 1151.4 (112.6) mL for total brain volume (see table 3). Each of the 20 volumes was normally distributed. The maximum/minimum volume ratio for total brain volume was 2.03. For the nine "other structures" in table 3, the maximum/minimum volume ratio ranged from a high of 12.4 for hippocampus to a low of 2.2 for internal capsule. As expected, volume was greatest for frontal lobe, followed in order by temporal, parietal, and occipital lobes. Of the smaller other structures, the combined medial structure was the largest, followed by the ventricles and the cingulate gyrus.

In linear regression modeling, all unadjusted and adjusted coefficients for the associations of PTL with ROI volumes were negative, indicating that brain volumes decreased as tibia lead increased (see table 3). For the unadjusted PTL coefficients, associations (i.e., $0.0001 < p < 0.05$) were observed for all ROIs examined except occipital white matter, corpus callosum, amygdala, hippocampus, entorhinal cortex, and cerebellum. After adjustment for confounders (see table 3 footnote), PTL exhibited a significant ($p < 0.05$) or borderline significant ($0.05 \leq p < 0.10$) association with nine ROIs (see table 3). Figure 1 shows

Table 4 *Profile of all former organolead workers by whether or not both MRI and X-ray fluorescence (XRF) tibia lead measures were obtained*

Variable	Completed MRI and XRF tibia lead measures		
	Yes	No	*p* Value for difference*
Number	532	447	
Age, y, mean (SD)	56.1 (7.7)	56.9 (8.3)	0.12
Exposure duration, y, mean (SD)	8.7 (9.8)	7.2 (9.2)	0.02
	n = 525	n = 412	
White race/ethnicity, %	92.3	91.5	0.65
Educational level, %			0.02
Less than high school	6.2	11.0	
High school graduate	58.8	52.5	
Some college	30.5	30.0	
College graduate	4.5	6.5	
Tobacco consumption, %			0.64
Never	29.9	27.3	
Current	20.2	20.4	
Previous	49.9	52.4	
Alcohol consumption, %			0.03
Never	4.0	2.0	
Current	73.5	69.1	
Previous	22.6	28.9	
Systolic blood pressure, mean (SD)	130.0 (13.4)	131.3 (15.8)	0.17
Diastolic blood pressure, mean (SD)	81.6 (8.5)	81.1 (9.3)	0.36
	n = 530	n = 427	
Hypertension†			0.07
Yes	38.0	43.7	
No	62.0	56.3	
Year enrolled			0.003
1994	20.7	21.7	
1995	39.4	49.0	
1996–97	7.2	6.7	
2001–03	32.8	22.6	
Current tibia lead, mean (SD)	14.5 (9.6)	15.7 (9.7)	0.10
Peak tibia lead, mean (SD)	23.9 (18.3)	27.1 (19.3)	0.02
Years since last exposure, mean (SD)	18.0 (11.0)	19.6 (11.5)	0.05

* *t* Test was used for testing differences in mean values; the chi-square test was used for categorical measures.
† Defined as systolic blood pressure > 140 or diastolic blood pressure > 90 or self-reported use of antihypertensive medications.

the relations of PTL with the six ROIs for which a significant association was observed.

The unadjusted and adjusted PTL coefficients in table 3 differed to some degree. These differences were primarily explained by adjustment for age, the strongest and most consistent predictor of volumes. Coefficients for other covariates (i.e., apolipoprotein E genotype and education) were negative (i.e., inverse relation) for almost all structures and significant for some. The association of education with ROI volumes was confined to those with less than a high school education. The coefficient for height was positive for most models and borderline or significant for 10 models, indicating that brain volume is directly related to height.

Discussion. This study supports our hypothesis that past exposure to organic and inorganic lead is associated with persistent global and region-specific brain lesions. Specifically, cumulative lead dose in former workers with past exposure to inorganic and organic lead is associated with an increased prevalence and severity of white matter lesions, total brain volume, and with smaller global and region- or structure-specific volumes. This finding is consistent with the previous association of longitudinal decline in neurobehavioral test scores to PTL, more than 15 years since last occupational lead exposure.[2] In our previous work using linear systems theory and an evaluation of these theories as applied to previous data (i.e., data on PTL and longitudinal change in neurobehavioral function), we concluded that the effect of lead in the brain was progressive.[3] Such an effect of lead could only be a consequence of a persistent or progressive structural lesion, not short-latency changes in brain neurochemistry or effects of lead on brain macromolecules. The findings from the current analysis are consistent with our previous hypothesis, with experimental evidence that lead can cause white matter damage, cell death, and changes to cellular architecture (i.e., synaptic density or other cellular connections),[4-12] and with our previous con-

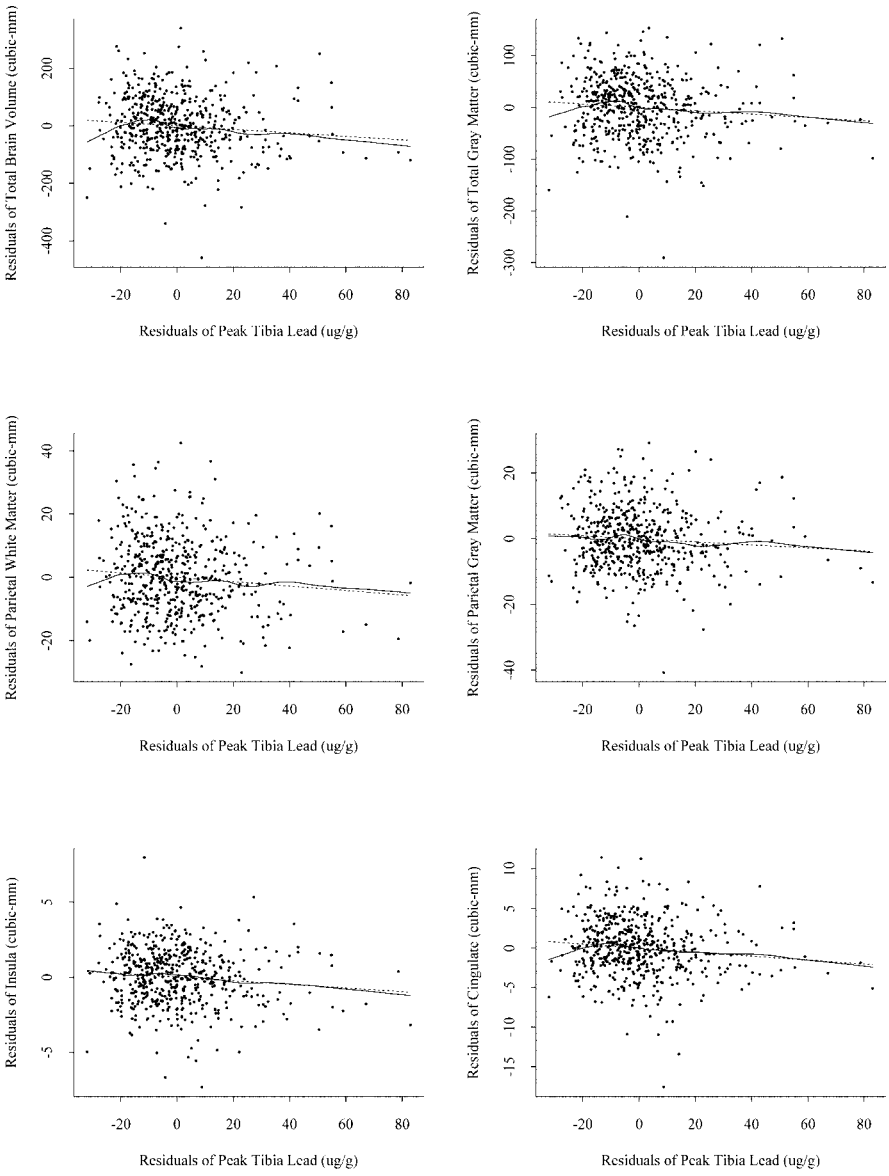

Figure 1. Linear regression (solid line) and Lowess (dashed line) plots for ROI volume residuals regressed on peak tibia lead (PTL as μg/g) residuals. Residuals reflect adjustment for covariates (details in table 3, footnote). Plots are presented for ROIs in table 4 for which a significant ($p < 0.05$) association was observed. Each plot exhibits a similar inverse relation, where the ROI residual decreases in value as the PTL residual increases. The Lowess plot indicates assumption of linearity for the regression model is reasonable.

clusions that a share of what is thought to be part of the spectrum of age-related changes in cognitive function in this cohort is likely to be explained by past exposure to lead.

The magnitude of the PTL effect on total brain volume is moderate and consistent with what was previously observed regarding cognitive function. In the prior longitudinal analysis of PTL and neurobehavioral decline, we compared the effect of individuals at the upper and lower ends of the interquartile range of PTL.[2] The lead effect was equivalent to what would be expected for 5 years of aging, given the average age of the cohort at the time data were collected. Analysis of the relation between PTL and total brain volume suggests a similar conclusion. Specifically, the 12.5 mL (i.e., 1.1% of total brain volume) decrease in total brain volume for an increase in PTL from the lower to the upper bound of the interquartile range is approximately equal to an effect we would expect for 5 years of aging, assuming that the mean cross-sectional association of each year of age with total brain volume predicts what would happen longitudinally, a reasonable expectation.[35]

In previous work,[1,2] PTL was associated with an accelerated and progressive decline in a diversity of cognitive domains, but with the strongest effects on verbal memory and learning, visual memory, and executive function. These specific effects may indicate disruption of widely distributed neural networks involved in the integration of functions and would be consistent with lesions to cortical association areas.[26,36] While we observed a PTL effect with total brain volume, total white matter volume, and total gray matter volume, the dose-response association with prevalence and severity of white matter lesions and the more selective paralimbic ROI effects are consistent with several possible hypotheses outlined below; none are mutually exclusive.

First, adult exposure to lead could accelerate age-associated changes in white matter. Myelination continues into the fifth and possibly sixth decade of life[37]

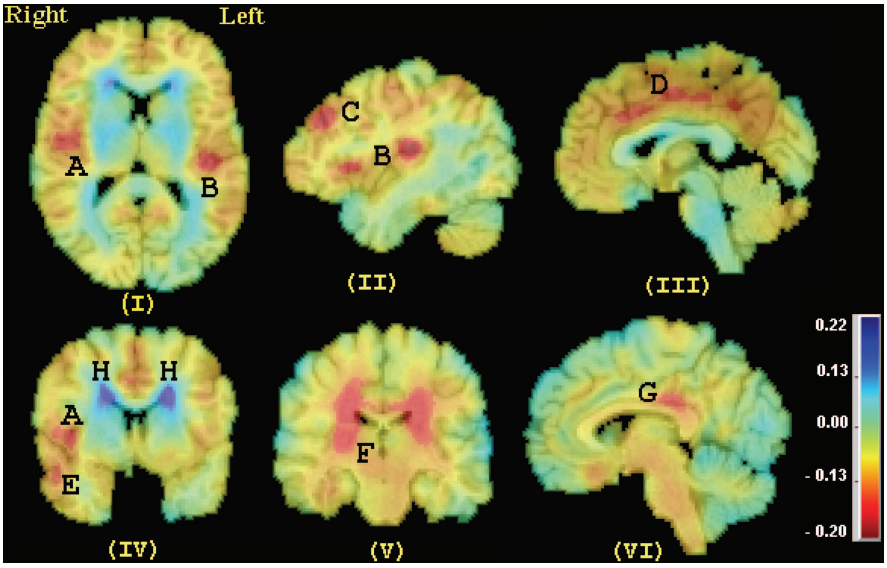

Figure 2. Spatial distribution of correlations between age-adjusted PTL and local tissue volume. The local tissue volume, in the vicinity of the respective image voxel, is shown for gray matter (GM) and abnormal white matter (WM) (I–IV) and WM alone (V–VI), superimposed on a reference brain image. Images are in radiology convention, i.e., right side of the image shows the left hemisphere. Color bar displays the correlation coefficients. Yellow and red indicate negative correlations (tissue atrophy), whereas blue indicates a positive correlation. For areas A to H above, parenthetical values are p values obtained after correction for multiple comparisons; the first p value is for cluster level significance and the second is for peak value significance after false discovery rate correction. (A) Right insula (0.005, 0.068); (B) left insula (0.317, 0.068); (C) left middle frontal gyrus (<0.0001, 0.068); (D) left cingulate gyrus (0.005, 0.068); (E) GM around right superior temporal sulcus (0.005, 0.068); (F) periventricular normal WM (left and right) (<0.0001, 0.006); (G) posterior corpus callosum (<0.0001, 0.006); and (H) abnormal periventricular WM (0.001, 0.002).

in selective brain regions (e.g., inferior temporal, prefrontal, and temporoparietal regions).[37,38] White matter produced later in life around cells with long but small caliber projections in cortico-cortical association areas[39] may be sensitive to oxidative stress and exogenous insults and a consequent pathophysiologic neurodegenerative cascade. The significant associations of PTL with the parietal white and gray matter, temporal white matter, and two relatively small paralimbic system structures (i.e., cingulate gyrus, insula) may suggest that lead accelerates an underlying age-associated process in selected brain regions.[38,39] It is noteworthy that no significant associations were observed in relatively large ROIs where myelination occurs early in life (e.g., occipital lobe and cerebellum) and where short axonal projections are relatively common.[39] We also did not find significant associations with limbic structures, contrary to our expectations. However, amygdala, entorhinal cortex, and hippocampus were the three smallest ROIs, possibly beyond the resolution of the volumetric method we used.

To address this limitation, we analyzed MRI data using a method that does not rely on a priori ROI definitions, and examines the entire brain in an unbiased region-by-region manner. In particular, this approach examines the spatial distribution of GM, white matter, and CSF throughout the entire brain, and determines regions where atrophy is associated with PTL. High-dimensional cortical pattern matching and image warping was used to achieve spatial coregistration of all images into a stereotaxic (canonical) coordinate system, enabling evaluation of spatial patterns of decreased tissue density.[40] In figure 2, the positive correlations (in blue) of PTL are predominantly with periventricular white matter lesions. These positive correlations are the product of an artifact whereby white matter lesions appear darker than normal and fall in the gray matter intensity range; for white matter, positive correlations indicate an association of PTL with increased white matter lesion severity. After correction for multiple comparisons with Statistical Parametric Mapping software, the areas most strongly associated with age-adjusted PTL included the bilateral insula, left middle frontal gyrus and left cingulate gyrus, gray matter around the right superior temporal sulcus, the posterior corpus callosum, and periventricular white matter.

Second, given the overall pattern of findings, we hypothesize that the global association of PTL with total brain volume and larger ROIs (e.g., total parietal or temporal lobes) is a consequence of progressive changes evolving from lesions in more specific ROIs. The associations we have observed with PTL may be a consequence of vascular effects of lead. Adult lead exposure is associated with longitudinal elevation of blood pressure,[41] a finding in line with the relatively strong associations we observed between PTL and periventricular white matter lesions. Vascular risk factors, including elevated blood pressure, are consistently associated with neurodegenerative changes[42] including white matter disease.

Third, lead, and in particular organolead, may have a direct effect on neuronal cells. Neurofibrillary tangles have been observed in experimental studies of rabbits with acute organic lead exposure.[10] We do not have sufficient evidence to evaluate the relative importance of these hypotheses, to identify the specific locus of initiation of lead's effect on the adult human brain, nor to specify what physiologic events initiate the pathophysiologic process. Longitudinal

MRI data will help to refine understanding of the specific sites that are affected and the initiating events.

We do not believe the study findings are explained by selection bias. Among the 979 former workers who participated in this study since its inception, we obtained MRIs and tibia lead measures on 532. While there are numerous significant differences between the MRI group and the 447 subjects not included in the MRI analysis on potential confounding variables (i.e., table 4), the differences are only slight to modest in magnitude and thus unlikely to account for the observed associations. For the most part, we believe selective loss is associated with early participation in the study, loss of interest, participation fatigue, and possibly a higher disease burden. Recognizing that PTL levels were higher among former workers without MRIs, we performed two additional analyses to evaluate selection bias by MRI status. First, after controlling for covariates we found, on average, neurobehavioral test scores at the most recent study visit did not differ by MRI status. Second, we found that relations of PTL with neurobehavioral test scores did not differ in those with and without MRIs. We thus believe that selection by MRI status is unlikely to account for our observations.

We do not believe the study findings are explained by residual confounding. In a review of previous studies,[14-19,43-46] the strongest and most frequently reported predictors of white matter lesions or total brain volume were sex and age followed by blood pressure or hypertension, education, ApoE genotype, serum lipids, smoking history, and alcohol consumption. Other than serum lipids, we accounted for these risk factors in our analysis. Of these, education raised a concern as to whether it was linked to both pre-exposure intellectual ability and to total brain volume. While education level is associated with brain volume at an older age,[47] there is no evidence that links education level with brain volume at a younger age. We believe that a host of health behaviors and risk factors are likely to explain the relation of low education with lower brain volume among the elderly. The association we have observed with education in our study is primarily attributable to the 6% of study participants with less than a high school education. When we adjusted for age, education was not associated with either current or peak tibia lead. Moreover, while low education level was associated with total brain volume and other global structural measures, low education level was not associated with the three smaller volumes for which a significant association was observed with PTL.

Finally, we decided not to include blood pressure or hypertension in the final logistic or linear regression models. In previous work, we showed that the mean blood pressure in this cohort increased by 0.73 mm Hg for every SD increase in tibia lead from baseline to 3 years later.[20] We reasoned that since lead increases blood pressure, blood pressure may be in the causal pathway between lead and volumes. Inclusion of blood pressure measures in this cross-sectional analysis could spuriously decrease our estimates of the total effect of lead on volumes. Nonetheless, when blood pressure measures were included in the logistic models for white matter lesions, they had no affect on PTL coefficients. Adjusting for both systolic and diastolic blood pressure in the ROI models had no affect on the PTL coefficient for the association with the cingulate gyrus, insula, or the corpus callosum. However, for total brain volume and the larger white and gray matter volumes, the coefficients for PTL were reduced by an average of 9% after adjustment for both systolic and diastolic blood pressure. Of the four significant associations, one was reduced to borderline significance (i.e., for total gray matter volume, the PTL coefficient changed from -0.326 [$p < 0.04$] to -0.286 [$p < 0.07$]).

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