

Lead Exposure and Amyotrophic Lateral Sclerosis

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Background. Previous interview-based studies have suggested that exposure to neurotoxicants including metals might be related to ALS.

Methods. We evaluated the relation of lead exposure to ALS, using both biological measures and interviews, in a case-control study conducted in New England from 1993 to 1996. Cases (N = 109) were recruited at two hospitals in Boston, MA. Population controls (N = 256) identified by random-digit dialing were frequency-matched to cases by age, sex, and region of residence within New England.

Results. Risk of ALS was associated with self-reported occupational exposure to lead (odds ratio [OR] = 1.9; 95% confi-

dence interval [CI] = 1.1–3.3), with a dose response for lifetime days of lead exposure. Blood and bone lead levels were measured in most cases (N = 107) and in a subset of controls (N = 41). Risk of ALS was associated with elevations in both blood and bone lead levels. ORs were 1.9 (95% CI = 1.4–2.6) for each $\mu\text{g}/\text{dl}$ increase in blood lead, 3.6 (95% CI = 0.6–20.6) for each unit increase in log-transformed patella lead, and 2.3 (95% CI = 0.4–14.5) for each unit increase in log-transformed tibia lead.

Conclusions. These results are consistent with previous reports and suggest a potential role for lead exposure in the etiology of ALS.

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Key words: amyotrophic lateral sclerosis, motor neuron disease, lead, occupational exposure, x-ray fluorescence, biomarker.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting the motor neurons of the spinal cord and brain.¹ Degeneration of spinal motor neurons leads to muscular atrophy and weakness, whereas degeneration of motor neurons in the cerebral cortex leads to hyperreflexia. ALS is rapidly progressive; patients generally die within 2–5 years of onset. The annual incidence rate of ALS is 1–2 per 100,000. This rate increases with age and is greater in men than in women. Approximately 5–10% of ALS cases have a family history of ALS.

In general, the etiology of ALS remains unclear, although genetic factors are involved in the familial form. Approximately 20% of familial cases have mutations in the gene for Cu/Zn superoxide dismutase (SOD), a cytosolic enzyme involved in detoxification of reactive oxygen species.^{2,3} Oxidative stress may also play

a role in sporadic ALS, although SOD mutations are rarely found in this form of the disease.^{2,3} Environmental exposures have also been considered as potential causes of ALS. Existing evidence has most clearly suggested a role for exposure to heavy metals, particularly lead, although electromagnetic fields and chemical exposures including pesticides and solvents have also been implicated.¹

We conducted a case-control study of ALS in New England from 1993 to 1996. The primary purpose of the study was to evaluate the relationship of lead exposure to ALS. We collected information on occupational, residential, and recreational exposure to lead using a structured interview. In addition, we measured blood and bone lead levels, the latter via *in vivo* K x-ray fluorescence (K-XRF).⁴ To our knowledge, no previous studies have reported bone lead levels in ALS cases measured with this technique.

Methods

Population

We recruited ALS cases between 1993 and 1996 from two major referral centers in New England: the Neuro-muscular Research Unit at New England Medical Center and the Neurophysiology Laboratory at Brigham and Women's Hospital. A board-certified neurologist (T. L. M. or J. M. S.) evaluated potential cases. Diag-

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nosis of ALS was based on criteria published by the World Federation of Neurology.⁵ Patients were eligible to participate if they had received an initial diagnosis of ALS within the 2 years before enrollment, if they lived in New England at least 50% of the year, if they spoke English, and if they were mentally competent. Of 223 sequential ALS cases evaluated, 22 were ineligible because their initial diagnosis occurred more than 2 years before enrollment, 31 were ineligible because of residence outside New England, 9 were ineligible because they did not speak English or were cognitively impaired, and 6 died before they could be enrolled. We encouraged ALS patients who had difficulty speaking to bring a relative or friend to the interview to help interpret their speech, and efforts were made to accommodate patients' other physical difficulties; nevertheless, some patients were too sick to participate. Of 154 eligible cases, 7 declined to participate because they were too sick, 15 declined because travel to the study site was too difficult, 20 refused for other reasons, and 2 could not be contacted. The total number of cases who participated in the study was 110 (71% of those eligible). One case did not complete the entire questionnaire because of illness and was excluded from the analyses reported here.

Potential controls were eligible if they lived in New England at least 50% of the year, spoke English, and were mentally competent. In addition, potential controls were excluded if they had received a physician diagnosis of Alzheimer's disease or other dementia, Parkinson's disease or parkinsonism, ALS or other motor neuron diseases, neuropathy, or post-polio syndrome. We frequency-matched controls to cases in 18 strata defined by three age groups (30–55, 56–65, and 66–80 years), sex, and three telephone area code groups (Boston, 617; Eastern Massachusetts, 508; and New England, 413, 203, 401, 603, 802, and 207). We used area code as a surrogate for distance of the subject's residence from the hospital. Matching was three to one in the 6 Boston strata and two to one in the other 12; this design gave approximately 2.3 controls per case overall.

Population controls were found by random telephone screening based on a modified Waksburg method.^{6,7} A two-stage sampling procedure was used; in the first stage households were screened to identify potential participants, and in the second stage the potential participants were contacted. Households were assigned to one of the 18 strata before contact. We called 9,926 residential phone numbers and, of these, 8,655 (87%) were successfully screened to identify 435 potential controls. When contact was attempted, 60 potential controls proved to be ineligible on the basis of criteria listed above, 5 had died or were too sick to interview, and 16 could not be contacted. Thus, 354 eligible controls were contacted; of these, 270 (76%) were enrolled in the study. Fourteen controls did not complete the entire interview and were

excluded from the analyses reported here. Of the 84 eligible controls who declined to be interviewed, 36 agreed to answer six questions focused on demographic factors and lead exposure. When we compared these 36 refusers with the 270 eligible controls who participated in the study, there were no differences in age, sex, region, or the proportion who reported having had a job involving lead exposure.

The Institutional Review Boards of the National Institute of Environmental Health Sciences, New England Medical Center, Brigham and Women's Hospital, Survey Research Associates-Batelle (Durham, NC), and CODA (Durham, NC) approved the study. All participants gave informed consent.

Questionnaire

Information on demographic and lifestyle characteristics and on residential and occupational histories was collected using a structured interview administered by trained personnel. Cases were interviewed in person and controls by telephone. The median length of the interview was 76 minutes (range 30–227).

Variables describing cigarette smoking and alcohol use have been described previously.⁸ Current levels of physical activity were characterized by the variable *inactivity*, defined as hours per day spent sitting, lying down, or sleeping. To characterize occupational exposure to lead, we asked participants whether they had ever had a job in which they were exposed to lead fumes, dust, or particles 10 or more times. Participants who responded affirmatively were asked how many years in total and on how many days per year during those years they were exposed to lead. Lifetime days of lead exposure were calculated by multiplying these two variables; the product was then categorized into four levels (0, 1–399, 400–1999, and 2000+). We also reviewed occupational histories to identify jobs potentially involving lead exposure (eg, battery manufacture or reclamation, soldering, working with firearms or on a firing range, painting, or paint removal), and a variable was created for occupational lead exposure on the basis of this review. The review indicated that 70% of individuals who reported occupational lead exposure had in fact had jobs potentially involving lead exposure, whereas only 30% of those not reporting occupational lead exposure had had such a job. We also collected information on residential characteristics potentially involving lead exposure for each residence a participant had occupied for at least 2 years and on participation in recreational activities potentially involving lead exposure.

Measurement of Blood and Bone Lead

We invited all cases and a subset of the controls to come to the laboratory for collection of blood samples and measurement of bone lead. Only controls who lived

within 20 miles of the testing center were invited to participate in this portion of the study: 99 were invited, and 41 participated (41%). Blood samples were collected from 107 cases and 39 controls. Bone lead measurements were made on 104 cases and 41 controls. We used logistic regression models to compare the 41 controls with laboratory measurements either to the 215 controls without measurements or to the subgroup of the latter who were invited to have measurements but declined ($N = 58$); independent variables were considered individually. Compared with the 215 controls without laboratory measurements, the 41 controls with measurements were more likely to be male, to have more than a high school education, and to be inactive; they were also more likely to be from Boston by design. The two groups were similar in terms of age, smoking, alcohol use, and occupational exposure to lead (ever or duration). The 41 controls with laboratory measurements were similar in all respects examined to the 58 controls from the Boston region who were invited to have measurements but declined.

Blood lead was measured using graphite furnace atomic absorption spectroscopy. Bone lead was measured using *in vivo* K-XRF. Measurements were made at two sites—the midtibial shaft and the patella—with a K-XRF instrument (ABIOMED, Inc, Danvers, MA). These two bones consist mainly of cortical and trabecular bone, respectively, and thus represent the two main bone compartments.⁴ A technical description and validity specifications for this instrument have been published elsewhere.^{9–11} This instrument provides an unbiased estimate of bone lead levels (as micrograms of lead per gram of bone mineral). Negative estimates of bone lead concentration may be obtained when true values are close to 0.

Data Analysis

The relationship of lead exposure to ALS was analyzed by unconditional logistic regression. All models included the matching variables age, sex, and region. In models that used all study participants to analyze questionnaire data, we modeled age as a categorical variable using the matching categories. Age was strongly related to lead levels, especially in bone. Consequently, we modeled age as a continuous variable in models using the subset of participants with laboratory measurements to analyze blood and bone lead. Education was related to ALS, and so a dichotomous education variable (\leq high school *vs* $>$ high school) was included except where noted. Because current physical activity levels were influenced by case-control status and also related to blood and bone lead levels, the variable inactivity (defined above) was included in some models relating ALS to lead levels. We also investigated the effect of adjusting the lead-ALS relationship for other factors previously

reported to affect lead levels, including cigarette smoking, alcohol use, income, and occupational exposure to lead. Associations are estimated as odds ratios (ORs), 95% confidence intervals (CIs) are based on standard errors, and two-tailed *P*-values for trend are derived from likelihood ratio tests.

Initial models relating blood and bone lead to ALS used categorical lead variables, with cutpoints set to divide the groups approximately into tertiles. Subsequent modeling used continuous lead variables to increase the statistical power of the analysis. Blood lead levels below assay sensitivity were assigned a value of 0.5 $\mu\text{g}/\text{dl}$, one-half the detection limit of 1. To remove extreme skewness in bone lead distribution while accommodating the few negative values, we transformed lead concentrations from both the tibia and patella using $\log_2([\text{Pb}] + 32)$, where [Pb] is bone lead concentration in $\mu\text{g}/\text{gm}$; a similar transformation has previously been used.^{12,13} Mean lead levels adjusted for age (as a continuous variable), sex, and region were calculated using the general linear-models procedure.

Results

Characteristics of Study Participants

Compared with the entire group of controls, cases were more educated and had smoked more cigarettes, but alcohol use did not differ (Table 1). Cases were inactive—sitting, lying down, or sleeping—for more hours per day than controls (Table 1). Other characteristics, including race, marital status, income, and body mass index, did not differ (data not shown). Risk estimates were qualitatively similar whether cases were compared with the entire group of controls or with the subgroup with laboratory measurements, although ORs for the latter comparisons were less precise.

Association of ALS with Occupational, Residential, and Recreational Exposure to Lead

Self-reported occupational exposure to lead was associated with ALS (Table 2). More cases than controls reported ever having had a job involving exposure to lead, and there was a dose response for lifetime days of lead exposure. Similarly, data based on review of self-reported occupational histories also showed that ever having had occupational exposure to lead was associated with ALS (OR = 1.8; 95% CI = 1.0–3.1). ALS was not positively associated with ever having lived in a residence with characteristics that might increase lead exposure (Table 3), with ever having engaged in recreational activities that might increase lead exposure (Table 3), or with time spent in such residences or engaged in such activities (data not shown).

TABLE 1. Characteristics of Participants in a Case-Control Study of Amyotrophic Lateral Sclerosis Conducted in New England, 1993–1996

	Cases (N = 109)		All Controls (N = 256)			Controls with Laboratory Measurements (N = 41)			95% CI
	N	%	N	%	Adjusted OR	95% CI*	N	%	
Age (years)									
30–55	38	35	85	33	—	*	11	27	*
56–65	37	34	73	29	—	—	12	29	—
66–80	34	31	98	38	—	—	18	44	—
Sex									
Men	66	61	156	61	—	*	31	76	*
Women	43	39	100	39	—	—	10	24	—
Region									
Boston metropolitan area	32	29	101	39	—	*	34	83	*
Eastern Massachusetts	28	26	53	21	—	—	7	17	—
New England	49	45	102	40	—	—	0	0	—
Education									
>high school†	71	65	200	78	1.0	—	37	90	1.0
≥high school	38	35	56	22	2.0	1.2–3.3	4	10	4.2
Cigarette smoking (pack-years)									
0‡	32	29	107	42	1.0	—	17	42	1.0
1–10	23	21	45	18	1.7	0.9–3.3	4	10	2.6
11–30	25	23	50	20	1.7	0.9–3.2	10	24	1.0
31+	29	27	53	21	1.7	0.9–3.3	10	24	1.6
									Trend P = 0.71
Alcohol use (drinks per month 5 years before interview)									
0‡	17	16	41	16	1.0	—	4	10	1.0
1–30	61	56	162	63	0.9	0.5–1.8	27	66	0.7
31+	31	28	53	21	1.4	0.7–3.0	10	41	1.0
									Trend P = 0.89
Inactivity (hours per day) (mean ± SD)	16.9 ± 4.5		13.9 ± 3.4		1.2	1.2–1.3‡	15.0 ± 3.3		1.2

Adjusted odds ratios (OR) and 95% confidence intervals (CI) comparing cases either with all controls or with controls with laboratory measurements were calculated by logistic regression with adjustment for age, sex, region, and education. Results for all controls have previously been published⁸; they are presented here for comparison. SD = standard deviation.
 * No ORs were calculated for the matching factors age, sex, and region.
 † Reference category.
 ‡ ORs were calculated for each hour per day increase in inactivity.

TABLE 2. Association of Amyotrophic Lateral Sclerosis (ALS) with Self-Reported Occupational Exposure to Lead in a Case-Control Study of ALS Conducted in New England, 1993–1996

	Cases		Controls		Adjusted OR	95% CI
	N	%	N	%		
Ever had a job involving exposure to lead fumes, dust, or particles 10 or more times						
No*	67	66	193	78	1.0	
Yes	35	34	54	22	1.9	1.1–3.3
Lifetime days of lead exposure						
0*	67	66	193	78	1.0	
1–399	8	8	17	7	1.6	0.6–3.9
400–1999	11	11	17	7	1.9	0.8–4.3
2000+	16	16	20	8	2.3	1.1–4.9
					Trend <i>P</i> = 0.02	

Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression with adjustment for age, sex, region, and education.

* Reference category.

Association of ALS with Blood and Bone Lead Levels

Three cases and no controls had blood lead levels greater than 10 µg/dl, five cases and one control had patella lead levels greater than 50 µg/gm, and two cases and no controls had tibia lead levels greater than 50 µg/gm. Blood and bone lead levels were strongly related to one another in both cases and controls. Combining data from the two groups, the Spearman correlation coefficient for blood lead and patella lead was 0.41, for blood lead and tibia lead 0.38, and for patella lead and tibia lead 0.54. In ALS cases, neither blood nor bone lead was correlated with time since diagnosis.

We first examined the association of ALS with lead levels using categorical lead variables (Table 4A). After adjusting for age, sex, region, education, and inactivity, ORs were elevated for blood, patella, and tibia, although estimates were imprecise. A dose-response relation was suggested for both blood and patella, although not for tibia. We conducted further analyses of the lead-ALS association were conducted using continuous lead variables (Table 4B). In blood, patella, and tibia, adjusted

mean lead levels were higher in cases than in controls, and adjusted ORs were elevated. Risk estimates from models including terms for education (Model 2) or for education and inactivity (Model 3) were essentially similar to models including only age, sex, and region (Model 1) (Table 4B). Further addition to Model 3 (Table 4B) of potential confounders, including cigarette smoking (ever smoked, pack-years), alcohol use (ever use, drinks per month), or income (≤\$30,000 vs >\$30,000), had little effect on the association of ALS with blood or bone lead (OR = 1.9 for all models for blood lead, 3.6–5.3 for patella lead, and 2.0–2.3 for tibia lead). Adjusting for occupational lead exposure had little effect on blood or patella lead (OR = 1.8 and 3.4, respectively) but weakened the association of tibia lead with ALS (OR = 1.3). The association of blood and bone lead with ALS was not changed by excluding from the analysis 12 cases whose initial diagnoses were more than a year old (OR for blood = 1.8, OR for patella = 4.4, and OR for tibia = 2.6) or seven cases and one control with a family history of ALS (OR for blood = 2.0, OR for patella = 3.8, and OR for tibia = 2.0).

TABLE 3. Relation of Residential Characteristics and Recreational Activities to Amyotrophic Lateral Sclerosis (ALS) in a Case-Control Study of ALS Conducted in New England, 1993–1996*

	Cases		Controls		Adjusted OR	95% CI
	N	%	N	%		
Residential characteristics						
Residence built before 1940	86	95	204	96	0.7	0.2–2.2
Residence located in a city with more than 500,000 inhabitants	38	40	88	41	1.0	0.6–1.8
Residence located on a street with traffic every few minutes	84	86	178	79	1.6	0.8–3.1
Residence located within 1 mile of a freeway	44	45	134	60	0.6	0.3–0.9
Residence located within 1 mile of a battery plant	3	3	8	4	0.9	0.2–3.5
Recreational activities						
Hunt	25	23	57	22	0.9	0.5–1.7
Shoot skeet, trap, or targets	37	34	72	28	1.5	0.9–2.5
Shoot on indoor range	17	16	47	18	0.9	0.5–1.6
Cast bullets, reload ammunition	8	7	20	8	1.0	0.4–2.3
Fish using lead weights	56	51	141	55	0.8	0.5–1.3
Glaze pottery or other ceramics	13	12	42	16	0.6	0.3–1.2
Solder	26	24	79	31	0.7	0.4–1.2

* Only subjects with residential information available for ≥80% of lifetime were included in residential analyses; total N = 91–98 cases and 213–226 controls. Adjusted odds ratios and 95% confidence intervals were calculated by logistic regression with adjustment for age, sex, region, and education.

TABLE 4. Association of Blood and Bone Lead with Amyotrophic Lateral Sclerosis (ALS) in a Case-Control Study of ALS Conducted in New England, 1993–1996

A. Lead as a Categorical Variable	Cases		Controls		Adjusted OR	95% CI
	N	%	N	%		
Blood lead ($\mu\text{g}/\text{dl}$)						
<1–2*	20	19	19	49	1.0	
3–4	44	41	11	28	14.3	3.0–69.3
5–14	43	40	9	23	24.5	4.3–139.3
Patella lead ($\mu\text{g}/\text{gm}$)						
–4–9*	27	26	14	34	1.0	
10–20	40	38	14	34	2.1	0.6–7.4
21–107	37	36	13	32	2.8	0.6–12.4
Tibia lead ($\mu\text{g}/\text{gm}$)						
–7–7*	21	20	14	34	1.0	
8–14	35	34	12	29	1.6	0.5–5.6
15–61	48	46	15	37	1.3	0.4–4.9

B. Lead as a Continuous Variable	Cases		Controls		Adjusted OR	95% CI
	Mean \pm SE†		Mean \pm SE†			
Blood lead ($\mu\text{g}/\text{dl}$)						
Model 1	5.2 \pm 0.4		3.4 \pm 0.4		1.8	1.3–2.4
Model 2					1.7	1.2–2.2
Model 3					1.9	1.4–2.6
Patella lead ($\mu\text{g}/\text{gm}$)						
Model 1	20.5 \pm 2.1		16.7 \pm 2.0		3.8	0.8–17.6
Model 2					2.3	0.4–12.2
Model 3					3.6	0.6–20.6
Tibia lead ($\mu\text{g}/\text{gm}$)						
Model 1	14.9 \pm 1.6		11.1 \pm 1.6		3.5	0.7–18.4
Model 2					2.3	0.4–13.2
Model 3					2.3	0.4–14.5

Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression. All models included age (as a continuous variable), square root of age, sex, and region. Models for categorical lead variables also included education (> high school vs \leq high school) and inactivity (hours per day spent sitting, lying down, or sleeping). For continuous lead variables, ORs were calculated for each unit increase in blood lead or in log-transformed bone lead. Model 1 included only age, sex, and region; Model 2 also included education; and Model 3 also included education and inactivity. SD = standard deviation; SE = standard error.

* Reference category.

† Means were adjusted for age (as a continuous variable), square root of age, sex, and region using the general linear models procedure. Values for 60-year-old men from Boston are presented.

Discussion

In this study, we found that increased risk of ALS was related to self-reported occupational lead exposure, although not to residential or recreational exposure. ALS was also associated with elevations in both blood and bone lead levels, although these results were imprecise.

Many previous studies have considered the hypothesis that lead exposure is a risk factor for ALS. The original description of ALS by Aron in 1850, which noted a connection of ALS with lead exposure (cited by Kasarkis¹⁴), was followed by case reports of ALS-like syndromes occurring after lead exposure.^{15–20} Subsequently, this issue was examined in nine case-control studies,^{21–29} all but one²⁴ of which found some evidence for an association of ALS with lead exposure estimated using questionnaire data. The four most recent studies, which involved more detailed exposure assessment, all found associations of ALS with self-reported occupational lead exposure, with ORs ranging from 1.9 to 5.7.^{26–29} Occupations potentially involving lead exposure, such as welding, have also been associated with ALS risk.^{26,27,30–32}

Although most case-control studies, including ours, found self-reported occupational lead exposure to be

associated with ALS, McGuire *et al*²⁹ reported that lead exposure based on expert evaluation of self-reported occupational histories by a panel of industrial hygienists was not associated with ALS (OR = 1.1), suggesting that recall bias might explain findings with self-reported data. In our study, occupational lead exposure based on a less formal review of self-reported occupational histories was associated with ALS; the OR was similar to that for self-reported lead exposure. Moreover, ALS was not associated with overall exposure to other neurotoxins, including mercury and pesticides. Thus recall bias may not entirely account for our findings. In contrast to occupational exposure, neither residential nor recreational exposure to lead was associated with ALS. Because these exposures are generally smaller and more difficult to characterize than occupational exposure, their effects may have been obscured by misclassification.

Previous studies of lead levels in blood, cerebrospinal fluid, or soft tissue of ALS patients are inconsistent. Studies from one group reported that ALS cases had higher lead levels than controls in plasma,³³ cerebrospinal fluid,³⁴ and muscle,³⁵ and another group found an elevation in spinal cord.³⁶ Other studies were nega-

tive.³⁷⁻⁴⁰ All of these studies have limitations including small size (21 cases at most), possible confounding by uncontrolled factors affecting lead levels, and potential bias introduced by use of controls with other neurologic diseases. One small study (N = 25) examined bone lead levels in ALS cases, using biopsy specimens from the iliac crest.²¹ Although lead levels were similar in cases and controls, the results are difficult to interpret because many cases had previously undergone chelation, and the chemical method used to measure lead (polarography) was of uncertain accuracy and is no longer in use.

Several factors related to blood and bone lead levels, including age, sex, cigarette smoking, alcohol use, and education,⁴¹⁻⁴³ are potential confounders of the lead-ALS relationship. ALS is associated with age, sex, and cigarette smoking, although not with alcohol use.^{1,8,44} In the present study ALS was also related to education, and, not surprisingly, to current levels of physical activity. However, with the exception of age, these factors did not appear to confound the relation of ALS with lead. Because age was the strongest predictor of bone lead in the present study, consistent with previous reports,^{12,13} our data may not be adequate to separate the effects of age and bone lead on ALS.

We found that the association of ALS with blood lead was strongest and most consistent, followed by the association with patella lead, whereas the association with tibia lead was less robust. The half-life of lead is progressively longer in blood (1 month), trabecular bone such as patella (3-5 years), and cortical bone such as tibia (15-25 years); blood lead is therefore considered to reflect recent exposure, and bone lead, especially cortical bone lead, to reflect cumulative exposure.⁴⁵ Studies in both humans^{46,47} and transgenic mice^{48,49} indicate that the loss of motor units in ALS is rapid, suggesting that disease onset might be abrupt and could potentially be related to recent rather than past or chronic exposure. Thus, a relation of blood lead to ALS is biologically plausible. Even so, our data suggest a surprisingly strong association with only small elevations in blood lead. In adults without current occupational lead exposure, patella lead is a major source of blood lead,¹³ perhaps accounting for the weaker but still important relation of ALS with patella lead. Tibia lead may constitute a slowly emptying reservoir only indirectly related to ALS. On the other hand, our data may simply not be adequate to demonstrate a relation with cumulative lead exposure reflected by tibia lead levels.

In the absence of external exposure, blood lead levels are determined by bone lead levels plus factors affecting mobilization of lead from bone. These factors may differ in ALS patients and controls. Certainly the decline in physical activity in ALS patients would promote bone demineralization. However, there may also be preexisting differences in lead metabolism, related for example

to a polymorphism in delta-aminolevulinic acid dehydratase⁵⁰ or to other genetic differences. Nongenetic factors promoting release of bone lead into blood may increase with age, perhaps contributing to the association of ALS with age.

A role for lead in ALS pathophysiology is plausible. In familial ALS, 20% of cases have a mutation in the SOD gene.⁵¹ Rather than reducing the normal protective function of SOD, these mutations appear to lead to a gain of toxic function, possibly involving oxidative damage to mitochondria or neurofilaments.^{2,3} Oxidative stress may also play a role in sporadic ALS.^{2,3} The pathophysiology of ALS may also involve excitotoxicity or mitochondrial dysfunction.^{2,3} All of these mechanisms likely involve impaired intracellular calcium homeostasis.³ Lead neurotoxicity depends on many of the same mechanisms. Lead can substitute for calcium in many intracellular reactions,^{52,53} lead damages mitochondria,^{52,54} lead causes oxidative damage to neural tissue,⁵⁵ and lead can amplify glutamate excitotoxicity.⁵⁶

Selection bias may have influenced our results. The low participation rate for population controls in the laboratory portion of the study is a particular concern. Controls with laboratory measurements were more likely to be male, inactive, and from Boston, and to have at least a high school education compared with those without measurements, but other characteristics of the groups did not differ, including self-reported occupational lead exposure. Some of the differences between the two groups (eg, being male or from Boston) would likely be associated with higher lead levels in the controls with measurements, which would tend to minimize rather than exaggerate the association of ALS with lead. Moreover, associations of ALS with blood and bone lead were observed after adjusting for all factors that differed between the groups. It is reassuring that potential controls who declined to participate in the overall study but answered the six basic questions reported a prevalence of occupational lead exposure similar to those who did participate; however, a majority of the nonparticipating controls did not respond to these questions. Cases were ascertained at two medical centers in Boston and therefore were likely not representative of the entire population of ALS cases in New England, creating another potential source of selection bias.

It is also possible that elevated blood lead levels are a result of ALS, although this seems an unlikely explanation of the elevation in bone lead levels. There are several arguments against reverse causality. Neither blood nor bone lead was correlated with time since diagnosis of ALS, and results were not changed by excluding cases with diagnoses more than 1 year old. A potential mechanism underlying reverse causality might be a decline in physical activity levels in ALS cases, leading to bone demineralization and release of lead

from bone into blood. However, the associations of blood and bone lead with ALS were not appreciably changed by adjusting for current physical activity levels.

The major strength of our study is the evaluation of lead exposure using both biological measurements and questionnaire data. Although neither approach is without problems, associations with ALS were found with both. Previous studies have suggested that lead exposure is associated with ALS, and a role for lead in ALS pathophysiology is plausible. Thus, the hypothesis that lead exposure plays a role in the etiology of ALS deserves further consideration.

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