ApoE Genotype, Past Adult Lead Exposure, and Neurobehavioral Function

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Our objective in this study was to determine if the known relation between tibia bone lead levels and neurobehavioral test scores are influenced by the apolipoprotein E (ApoE) genotype. We collected data on 20 neurobehavioral tests in 529 former organolead workers who had an average of 16 years since last occupational exposure to lead. We used linear regression to model the relations between each of 20 neurobehavioral test scores and tibia lead, a binary variable for ApoE genotype (i.e., at least one $\varepsilon 4$ allele vs. none), and an interaction term between tibia lead and the binary term for ApoE genotype. At the time of testing, former lead workers were an average of 57.6 years of age; 82% were younger than 65 years. In regression analysis, we observed one statistically significant and one borderline significant coefficient for ApoE genotype alone. Coefficients for the ApoE and tibia lead interaction term were negative in 19 of the 20 regression models. This indicates that the slope for the relation between tibia lead and each neurobehavioral test was more negative for individuals with at least one ϵ 4 allele than for those who did not have an ϵ 4 allele. Four of 19 negative coefficients for the interaction term were statistically significant (digit symbol, Purdue pegboard assembly, Purdue pegboard-dominant hand, complex reaction time); another three of the remaining 16 coefficients (symbol digit, trail-making A, Stroop) were borderline significant (i.e., p < 0.10). This study suggests that individuals may vary in susceptibility to the long-term effects of lead on the central nervous system (CNS). In particular, the persistent CNS effect of lead may be more toxic in individuals who have at least one ApoE-E4 allele. Key words: apolipoprotein E, bone lead, cognitive function, neurobehavioral tests, X-ray fluorescence. Environ Health Perspect 110:501-505 (2002). [Online 2 April 2002] http://ehpnet1.niehs.nih.gov/docs/2002/110p501-505stewart/abstract.html

In a previous cross-sectional study (1), we showed that past (i.e., an average of 16 years before study enrollment) occupational exposure to organic and inorganic lead, as measured in tibia by X-ray fluorescence, was associated with poorer performance on neurobehavioral tests. We observed a stronger association in the domains of manual dexterity, executive ability, and verbal memory. Function in these same domains is also declining over time in relation to tibia lead (2). In this population, the effect of adult lead exposure on central nervous system (CNS) function appears to be progressive, possibly mediated by permanent CNS changes involving cellular damage.

The specific mechanisms mediating the relation of past adult lead exposure on the adult CNS are not known. Nonetheless, these findings and other evidence support the notion that the effect of past exposure to lead in adults is progressive and, as a consequence, must be mediated by permanent CNS changes that are likely to involve cellular damage and associated pathophysiologic responses. In this regard, epidemiologic and pathophysiologic evidence indicates that the short- and long-term consequences of physical insults to the CNS (e.g., traumatic brain injury) are influenced by whether or not an individual has at least one $\varepsilon 4$ allele for the apolipoprotein E (ApoE) gene (3-7).

We considered whether the effect modification of the *ApoE*- ε 4 allele that has been observed with traumatic brain injury was generalizable to the changes induced by past exposure to lead. To test this hypothesis, we used data from a previous cross-sectional study of former organolead manufacturing workers (1) on whom *ApoE* genotyping had been completed. We specifically evaluated whether tibia lead levels have a larger influence on neurobehavioral test scores in subjects with the *ApoE*- ε 4 allele compared to those without the allele.

Methods

Study design and overview. We derived data from a longitudinal study of CNS and peripheral nervous system function in former employees of a chemical manufacturing facility in the eastern United States that, in the past, produced tetraethyl and tetramethyl lead. The methods are described in detail elsewhere (1,2).

Here we report on a cross-sectional analysis of data from an original cohort of 544 individuals for whom we measured cognitive function and tibia lead. We obtained *ApoE* genotype on 529 of the 544 cohort members. 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.

Selection and recruitment of study subjects. Study participants worked in a plant that manufactured a broad range of chemical products; the present study focused on individuals who were ever employed in the plant area that manufactured tetraethyl lead from 1923 to 1991 and tetramethyl lead from 1960 to 1983 (8). None of the subjects recruited for this study were occupationally exposed to lead at the time of enrollment. Subjects were eligible for recruitment if they were ever employed in the facility on or after 1 January 1950, were male, and were between 40 and 70 years of age in 1995. Of an estimated 968 eligible subjects, we enrolled 73% (n = 703) in the study.

Data collection. We read the consent statement to subjects who completed a clinic visit. After obtaining consent, we collected data in the following order: Symptom CheckList-90 (SCL-90) (9), blood pressure, height, weight, an initial interview (i.e., demographics, problems with vision or hearing, medical history, use of nonsteroidal anti-inflammatory drugs, history of head, hand, or finger injuries, physical activity level), neurobehavioral testing, the Center for Epidemiologic Study Depression scale (CES-D) (10), the Scandinavian Questionnaire 16, and an exit interview (i.e., occupational history, smoking history, alcohol consumption history). The neurobehavioral test battery has been described in detail elsewhere (1,2). We completed the initial visit by obtaining two 10-mL blood specimens by venipuncture. In a separate visit, we also measured tibia lead.

Tibia lead and ApoE *genotype.* We measured tibia lead for 30 min (true time) using

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¹⁰⁹Cd to fluoresce the k-shell X ray of lead. We measured lead at the left midtibia shaft (11-14). Because of large interindividual differences in time since last occupational lead exposure, we used years since last exposure to estimate tibia lead levels at the termination of lead exposure, termed peak tibia lead. We extrapolated current tibia lead levels using a clearance half-time of lead in tibia of 27 years (15), assuming first-order (monoexponential) clearance from tibia (1). We have shown that peak tibia lead is the strongest predictor of the cognitive effects of lead and that it offers the best approximation of the area under the exposure curve reflected by the duration of exposure multiplied by mean exposure intensity (16).

We completed *ApoE* genotyping using the method of Hixson and Vernier (17). We carried out reactions in a total volume of 50 µL with 2.5 µM primer, 0.2 µM deoxyribonucleoside triphosphate, 1.5 mM MgCl₂, 0.25 µL (1.25 U) of Taq polymerase, and 10% dimethyl sulfoxide. Cycling was 95°C for 30 sec, 60°C for 30 sec, and 71°C for 1 min for 40 rounds. The polymerase chain reaction product was digested by *Hha*I and run on 4% agarose gels for genotype determination. Primers were F4-ACAGAATTCGCCCCGGC-CTGGTAC and F6-TAAGCTTGGCAC-GGCTGTCCAAGGA.

Statistical analysis. We previously showed that peak tibia lead is consistently associated with poorer performance on neurobehavioral tests (1). We limited the present analysis to evaluating whether *ApoE* genotype modified the relations between peak tibia lead and neurobehavioral test scores using data from a single visit (i.e., at cross section).

We examined neurobehavioral measures for outliers attributable to stroke, head injury, and other organic conditions or physical impairments, and evaluated variable distributions for normality. We standardized all neurobehavioral test scores for directionality so that a negative coefficient in the model indicates that performance on a neurobehavioral test was worse with increasing lead exposure or dose. Distributions of scores for five neurobehavioral tests evidenced departures from normality. Before modeling, we transformed the Stroop test as the square root, and log-transformed four other tests (trail-making A and B, choice reaction time, and SCL-90 Global Severity Index).

We used generalized linear models (18) to examine the relations of peak tibia lead, ApoEgenotype (at least one $\varepsilon4$ allele vs. none), and the cross product of peak tibia lead and ApoEgenotype with neurobehavioral test scores. We could not address questions about one versus two $\varepsilon4$ alleles because only six individuals had two $\varepsilon4$ alleles. Other variables in the model included age (linear and quadratic terms), race (white vs. other race/ethnicity), education (less than high school, high school diploma, some college, college degree, graduate training), testing technician (four technicians), duration of occupational lead exposure (in years), and depression status as measured by the CES-D (< 16, \geq 16) (10). We excluded individuals if they had a recent history (i.e., stroke, hand injury for selected tests) that could influence performance on neurobehavioral tests.

Results

Demographic and exposure profile. The demographic and exposure profile of the population has been previously described (1,2). In this section, we focus on differences by *ApoE* genotype. The most common genotype variant among the 529 study participants was $\epsilon 3\epsilon 3$ (67.1%), followed in order by $\epsilon 3\epsilon 4$ (18.0%), $\epsilon 2\epsilon 3$ (10.4%), $\epsilon 2\epsilon 4$ (3.2%), $\epsilon 4\epsilon 4$ (1.1%), and $\epsilon 2\epsilon 2$ (0.2%). For the analysis, we categorized individuals as not having an $\epsilon 4$ allele (n = 411; 77.7%) and having at least one $\epsilon 4$ allele (n = 118; 22.3%). We

observed no differences between individuals with and without an *ApoE*- ϵ 4 allele for age, education, time since last exposure, or tibia lead levels (Table 1). As expected (*19,20*), we observed a statistically significant difference in *ApoE*- ϵ 4 allele prevalence by race (p = 0.003); 43% of nonwhites, predominantly African Americans, had at least one *ApoE*- ϵ 4 allele compared to 21% of whites. Individuals with at least one *ApoE*- ϵ 4 allele tended to work for a longer period of time in the organolead facility compared to those without the ϵ 4 allele (p = 0.06).

ApoE genotype, peak tibia lead, and neurobehavioral function. In a previous analysis (1), peak tibia lead coefficients were negative for all 20 neurobehavioral tests. Higher peak tibia lead levels were significantly (p < 0.05) associated with poorer performance on 11 of the 20 tests.

In the present analysis, we added a binary variable for *ApoE* genotype (i.e., presence or absence of at least one $\varepsilon 4$ allele) to the model in the absence of tibia lead. Twelve of the 20 coefficients for *ApoE* genotype were negative

Table 1. Percentage distributions and mean ± SD of selected study variables by *ApoE*-ɛ4 allele status in 529 former organolead workers in whom tibia lead and neurobehavioral function were measured in 1996–1997.

| | Number of <i>ApoE</i> -ε4 alleles | | | |
|---|-----------------------------------|-------------------|--|--|
| | None | At least one | | |
| Variable/category | (<i>n</i> = 411) | (<i>n</i> = 118) | | |
| Age* (years) | | | | |
| 40-49 | 18.0 | 16.1 | | |
| 50–54 | 20.7 | 19.5 | | |
| 55–59 | 22.6 | 22.0 | | |
| 60–64 | 22.2 | 15.3 | | |
| 65–69 | 12.4 | 17.8 | | |
| ≥ 70 | 4.1 | 9.3 | | |
| Mean ± SD | 57.3 ± 7.4 | 58.5 ± 7.8 | | |
| Education* | | | | |
| Less than high school | 7.5 | 8.5 | | |
| High school diploma or equivalent | 58.4 | 61.9 | | |
| Some college or technical school | 29.2 | 24.6 | | |
| College degree | 4.9 | 5.1 | | |
| Race** | | | | |
| White | 95.1 | 87.3 | | |
| Not white | 4.9 | 12.7 | | |
| Exposure duration* (years) | | | | |
| <1 | 22.4 | 17.8 | | |
| 1-2.99 | 23.1 | 19.5 | | |
| 3-4.99 | 12.9 | 9.3 | | |
| 5-9.99 | 14.4 | 25.4 | | |
| | 27.2 | 28.U | | |
| IVIEAN ± SD | 8.1 ± 9.7 | 8.8 ± 10.0 | | |
| | 16.1 | 14.4 | | |
| | 10.1 | 14.4 | | |
| J-9.99 10 14 00 | 10.3 | 20.3 0 5 | | |
| 10−14.55 < 15 | 51 7 | 56.8 | | |
| ≥ 10 Moon $\pm 9D$ | 17 9 ± 11 6 | 17 Q ± 11 / | | |
| Peak tibia lead* (ug Ph/g hone mineral) | 17.5 ± 11.0 | 17.5 ± 11.4 | | |
| | 10 5 | 51 | | |
| 5-9 99 | 11.0 | 14.5 | | |
| 10-14.99 | 13.4 | 9.4 | | |
| 15–19.99 | 16.6 | 14.5 | | |
| ≥ 20 | 48.5 | 56.4 | | |
| Mean ± SD | 23.1 ± 16.9 | 26.2 ± 19.1 | | |

*The difference by ApoE genotype was not statistically significant (p > 0.10), as assessed by t-test for continuous variable comparisons or chi-square for categorical variable comparisons. ** $p \le 0.01$.

(model I, Table 2); one of the negative coefficients was statistically significant (i.e., Stroop), and one other was borderline significant (i.e., trail-making B; p < 0.10). Coefficients for *ApoE* genotype were not substantially changed after adding peak tibia lead to each of the 20 models (model II, Table 2). We subsequently added a cross-product term for *ApoE* genotype and peak tibia lead to each model. Nineteen of the 20 coefficients for the interaction term were negative (model III, Table 2); 4 of the 20 coefficients were significant (p < 0.05), and 7 had associated *p*-values < 0.10.

To interpret the magnitude of the effect from the interaction of ApoE- ϵ 4 with tibia lead, we compared the coefficients for the interaction term to the coefficient for tibia lead alone (i.e., slope for the relation between tibia lead and neurobehavioral test score) for the seven tests where we observed a statistically significant or borderline significant effect. *ApoE*- ϵ 4–positive status appears to increase the slope for the relation between tibia lead and neurobehavioral test scores by 1-fold (i.e., Stroop) to 4-fold (i.e., trail-making A), with a 2-fold increase as the median.

Discussion

Negative coefficients for the interaction term in this analysis indicate that peak tibia lead levels may have a greater adverse influence on neurobehavioral test scores in subjects with the ApoE- ϵ 4 allele compared to those without the allele. Previous studies have shown that ApoE genotype modifies the effect of physical insults on the long-term risk of dementia (3,6) and risk of dementia after stroke (21). The results of this study suggest that the effect-modifying pathophysiologic role of the ApoE- ϵ 4 allele in the CNS may be extended to include the long-term effects of adult exposure to lead.

The *ApoE* genotype appears to play a role in the acute and long-term recovery from physical insults. Elevated β -amyloid deposits have been observed in humans during the acute phase of recovery from traumatic brain injury (22,23), deposits that appear more pronounced in those with the *ApoE*- ϵ 4 allele (4). The ϵ 4 allele is also associated with delayed recovery from trauma-induced coma (5,7) and risk of dementia among individuals with a history of brain trauma (3,6), especially if the brain trauma occurs at a later age (3).

The data from this study suggest that ApoE genotype may modify the relation between peak tibia lead and neurobehavioral function. Specifically, slopes for the relations between peak tibia lead and neurobehavioral test scores were more negative (i.e., more adverse) for individuals with at least one ApoE-E4 allele. We observed the strongest and most consistent associations for the interaction term for measures of executive ability, a finding that differs to some degree from what we observed (1) for the main effect of tibia lead alone (i.e., manual dexterity followed, in order, by verbal memory and learning, and executive ability). These contrasting findings may reflect differences in the local anatomic pathophysiology induced by lead alone (e.g., due to the selective deposition of lead), by ApoE genotype alone (e.g., where Alzheimer-related pathology is likely to first emerge), or by the combination of lead and ApoE genotype (e.g., overlapping CNS regions).

The interaction between the ApoE- $\epsilon4$ allele and past exposure to lead suggested by our data is particularly noteworthy given that an average of 16 years had passed between the last time individuals were occupationally

Table 2. Linear regression coefficients^a (SE) for peak tibia lead, *ApoE* genotype (at least one ε4 allele vs. none), and the interaction between peak tibia lead and *ApoE* genotype as predictors of neurobehavioral test scores in former organolead workers, 1997.

| Domain/neurobehavioral test ^c | Model I | Model II | | Model III | | |
|--|---|---|---|--|--|--|
| | <i>ΑροΕ</i> -ε4 β (SE β) ^d | Peak tibia lead β (SE β) ^d | <i>ΑροΕ</i> -ε4 β (SE β) ^d | Peak tibia Lead β (SE β) ^d | <i>ΑροΕ</i> -ε4 β (SE β) ^d | Interaction term ^b β (SE β) ^d |
| Visuo-construction/visuo-perception | | | | | | |
| Block design (WAIS) | 0.784 (0.917) | -0.039 (0.026) | 0.933 (0.921) | -0.029 (0.029) | 1.877 (1.555) | -0.037 (0.049) |
| Rey complex figure, copy | 0.201 (0.452) | -0.015 (0.013) | 0.291 (0.454) | -0.005 (0.014) | 1.236 (0.765) | -0.040 (0.024) |
| Verbal intelligence | | | | | | |
| Vocabulary (WAIS-R) | 0.788 (1.000) | -0.063 (0.028)** | 1.010 (1.000) | -0.066 (0.031)** | 0.718 (1.688) | 0.012 (0.054) |
| Verbal memory and learning Serial digit learning Bey auditory verbal learning test | -0.867 (0.737) | -0.048 (0.021)** | -0.723 (0.739) | -0.045 (0.023) | -0.428 (1.248) | -0.012 (0.040) |
| Immediate recall Recognition | -0.474 (0.949) -0.217 (0.320) 0.125 (0.327) | -0.068 (0.026)** -0.016 (0.009)* -0.011 (0.009) | -0.149 (0.944) -0.193 (0.321) 0 187 (0 328) | -0.063 (0.030)** -0.014 (0.010) -0.006 (0.010) | 0.333 (1.587) -0.023 (0.542) 0.613 (0.552) | 0.019 (0.051) 0.007 (0.018) 0.017 (0.018) |
| Visual memory | 0.120 (0.027) | 0.011 (0.000) | 0.107 (0.020) | 0.000 (0.010) | 0.010 (0.002) | 0.017 (0.010) |
| Symbol digit (WAIS-R) | -0.300 (0.482) | -0.017 (0.014) | -0.216 (0.483) | -0.004 (0.015) | 0.946 (0.812) | -0.046 (0.026)* |
| Rey complex figure, delayed recall | 0.630 (0.538) | -0.025 (0.015)* | 0.720 (0.540) | -U.UI7 (U.UI7) | 1.477 (0.910) | -0.030 (0.029) |
| Digit symbol substitution | -0.729 (0.990) | -0.027 (0.028) | -0.587 (0.994) | 0.001 (0.031) | 2.149 (1.678) | -0.109 (0.054)** |
| Part A ^e Part B ^e | 0.570 (3.063) -0.058 (0.034)* | -0.068 (0.086) -0.001 (0.001) | 1.086 (3.077) -0.053 (0.034) | 0.013 (0.096) -0.001 (0.001) | 8.755 (5.174)* -0.013 (0.061) | -0.303 (0.164)* -0.002 (0.002) |
| Purdue pegboard, assembly | -1.049 (1.825) | -0.122 (0.050)** | -0.490 (1.819) | -0.074 (0.056) | 4.241 (3.058) | -0.189 (0.098)** |
| Manual dexterity | -0.341 (0.140) | -0.013 (0.004) | -0.313 (0.147) | -0.010 (0.003) | 0.014 (0.240) | -0.013 (0.000) |
| Finger-tanning | | | | | | |
| Dominant hand | -0 436 (0 731) | -0 033 (0 020)* | -0 252 (0 732) | -0.030 (0.023) | 0 086 (1 229) | -0 014 (0 040) |
| Non-dominant hand | -0 251 (0 651) | -0.045 (0.018)** | -0 136 (0 651) | -0.041 (0.020)** | 0.307 (1.094) | -0.018 (0.035) |
| Purdue pegboard | 0.201 (0.001) | 01010 (01010) | 0.100 (0.001) | 01011 (01020) | 0.007 (1.00 1) | 0.010 (0.000) |
| Dominant hand | 0.311 (0.521) | -0.036 (0.014)** | 0.441 (0.521) | -0.019 (0.016) | 2.127 (0.869)** | -0.068 (0.028)** |
| Non-dominant hand | -0.124 (0.516) | -0.046 (0.014)** | 0.023 (0.513) | -0.035 (0.016)** | 1.070 (0.861) | -0.042 (0.028) |
| Both hands | 0.010 (0.458) | -0.035 (0.013)** | 0.145 (0.456) | -0.028 (0.014)** | 0.843 (0.765) | -0.028 (0.025) |
| Psychomotor Complex reaction time (msec) ^e | -0.004 (0.020) | -0.001 (0.001) | -0.002 (0.020) | | 0.061 (0.033)* | -0.003 (0.001)** |
| | 0.001 (0.020) | 0.001 (0.001) | 0.002 (0.020) | 0.000 (0.001) | 0.001 (0.000) | 0.000 (0.001) |

Abbreviations: WAIS, Wechsler Adult Intelligence Scale; WAIS-R, Wechsler Adult Intelligence Scale-Revised.

^aAdjusted for age (linear and squared terms), race, education level, CES-D score (i.e., depression status), testing technician, and visit number. ^bCross-product term of peak tibia lead and *ApoE* genotype. ^cAll measures have been standardized so that a negative coefficient indicates that neurobehavioral test scores are worse as exposure or dose increases. ^dThe β coefficient from the regression model and its SE. ^eThese tests were transformed before modeling due to departures from normality. **p* ≤ 0.10; ***p* ≤ 0.05.

exposed to lead and enrollment in the study. If this interaction is real, it cannot be explained by an acute effect of lead. Rather, past lead exposure may have induced persistent (1,2) and progressive (2) changes, a view that is consistent with an effect-modifying role of ApoE genotype and the suspected biologic effects of organic and inorganic lead. Several possible mechanism may explain the observed interaction.

Triethyl lead, the first dealkylated metabolite of tetraethyl lead, inhibits oxidative phosphorylation, amino acid metabolism, glucose oxidation, phosphocreatine synthesis, and neurotransmitter metabolism (24). Triethyl lead also inhibits microtubule synthesis, leading to the clumping and shrinking of cells, with vacuolar degeneration and cellular necrosis (25). Tetraethyl lead is converted to inorganic lead in rat brain (26,27).

Lead appears to induce cell damage that could explain, in part, persistent morphologic changes observed in animal experimental studies. Glial fibrillary acidic protein, a cellspecific cytoskeletal intermediate filament protein used as a marker of number and size of astrocytes, increases in the hippocampus of animals dosed with either organic or inorganic lead (28-30). Moreover, morphologic changes in the CNS have been observed in the rat hippocampus even at low lead levels (31,32). Lead may have both a selective and a general effect on CNS function. After injection of tetraethyl lead in rabbits, degenerative changes and neurofibrillary tangles have been observed in the pyramidal cells of the frontal cortex and hippocampus (26,33,34). Evidence consistently shows that lead preferentially, but not exclusively, affects the prefrontal cerebral cortex, hippocampus, and cerebellum (35-47).

The neurobehavioral deficits that were associated with tibia lead levels directly differ to some degree from those that were significantly associated with the interaction term for tibia lead and ApoE. Ultimately, we are faced with the limitation that specific domains of function cannot be uniquely mapped to defined brain regions given the battery of neurobehavioral tests that we used. This limitation raises challenges in formulating a biologic rationale that accounts for the differences we have observed for tibia lead alone versus tibia lead and ApoE genotype. Imaging studies (i.e., volumetric and brain activity measures) in this population may be helpful in validating the neurobehavioral findings and providing the foundation for a more specific biologic rationale to support the interaction between the ApoE-E4 allele and past exposure to lead.

We did not observe a direct effect of *ApoE* genotype (i.e., without terms for a main

effect of peak tibia lead or for an interaction between lead and ApoE) on cognitive function. The absence of significant findings may be explained by the relatively young age of study participants. At the time of testing (for most subjects, we used the third study visit in this analysis, the time of bone lead measurement), individuals were, on average, 57.6 years of age with a range from 41 to 73 years; 82% of the population was < 65 years of age. This explanation is consistent with the relation that we observed between neurobehavioral test scores and the cross-product term for ApoE genotype and age. We observed significant (i.e., p < 0.05) or borderline significant (i.e., p < 0.10) negative coefficients for the interaction term for 10 of the 20 neurobehavioral tests. This finding indicates that a measurable effect of the ApoE- ε 4 allele on neurobehavioral function is detectable at older but not necessarily younger ages.

In conclusion, the results of this study support the notion that individuals may vary in susceptibility to the long-term effects of lead on the CNS. In particular, the persistent CNS effect of lead may be more toxic in individuals who have at least one *ApoE*-ɛ4 allele.

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