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Cognitive Deficits and Magnetic Resonance Spectroscopy in Adult Monozygotic Twins with Lead Poisoning

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Seventy-one-year-old identical twin brothers with chronic lead poisoning were identified from an occupational medicine clinic roster. Both were retired painters, but one brother (J.G.) primarily removed paint and had a history of higher chronic lead exposure. Patella and tibia bone lead concentrations measured by K-X-ray fluorescence in each brother were 5–10 times those of the general population and about 2.5 times higher in J.G. than in his brother (E.G.). Magnetic resonance spectroscopy (MRS) studies examined N-acetylaspartate:creatine ratios, a marker of neuronal density. Ratios were lower in J.G. than in his brother. Scores on neurocognitive tests that assess working memory/Executive function were below expectation in both twins. Short-term memory function was dramatically worse in J.G. than in his brother. These results demonstrate some of the more subtle long-term neurologic effects of chronic lead poisoning in adults. In particular, they suggest the presence of frontal lobe dysfunction in both twins, but more dramatic hippocampal dysfunction in the brother with higher lead exposure. The MRS findings are consistent with the hypothesis that chronic lead exposure caused neuronal loss, which may contribute to the impairment in cognitive function. Although a causal relation cannot be inferred, the brothers were genetically identical, with similar life experiences. Although these results are promising, further study is necessary to determine whether MRS findings correlate both with markers of lead exposure and tests of cognitive function. Nevertheless, the results point to the potential utility of MRS in determining mechanisms of neurotoxicity not only for lead but also for other neurotoxicants as well.

Key words: lead poisoning, magnetic resonance spectroscopy, monozygotic, neuropsychological tests, paint, twins. Environ Health Perspect 112:620–625 (2004). doi:10.1289/ehp.6687 available via http://dx.doi.org/ [Online 8 January 2004]

Case Presentation

J.G. and E.G. are 71-year-old monozygotic twins. Both are retired painters who worked in the Boston metropolitan area. The brothers worked together but performed different, well-defined tasks: J.G. removed paint by scraping, sanding, and heat treatment with an electric iron; E.G. predominantly painted but at times assisted in paint removal. J.G. smoked cigarettes and ate at work without washing his hands. E.G. smoked cigarettes until the early 1990s but reported that he was meticulous about washing his hands before eating at work. Both wore paper masks at work but did not use any sophisticated respiratory protective devices.

In 1984, J.G. developed chronic back pain, for which he was referred to a neurosurgeon. Because of his occupational history of painting, a blood lead (BPb) level was ordered and returned at 125 μg/dL. He was subsequently hospitalized and chelated with ethylenediamine tetra-acetic acid (EDTA). Since his chelation he has been followed in the Center for Occupational and Environmental Medicine at what is now the Northeast Specialty Hospital. J.G.’s other chronic health problems include hypertension, which E.G. has as well. Otherwise both brothers are healthy. Both E.G. and J.G. graduated from the same high school, served together in the Navy, and have worked together as painters for > 45 years. The combination of differential lead exposures in the context of complete genetic matching and similar childhood and adult environments provided a unique opportunity for assessment of central nervous system (CNS) effects of lead. Cognitive testing and magnetic resonance spectroscopy (MRS) studies were employed to determine differences that could be attributed to differential lead exposures.

Neurocognitive testing. Both brothers were tested on a battery of cognitive tests, including the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981) and the Mini-Mental State Exam (MMSE) (Folstein et al. 1975). A battery of specific tests designed to assess the domains of attention, executive function, verbal and language skills, visuospatial abilities, manual motor speed and dexterity, memory and behavior/personality was also performed.

J.G. underwent cognitive testing in both 1990 and 1999. Based on his reported academic history and his performance on tests of verbal and academic ability that are relatively impervious to the effects of CNS insults in adulthood, his premorbid verbal/language abilities were judged to be at the lower end of the average range and his visuospatial skills to be at the upper end of the average range. In

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We are indebted to the steadfast and patient cooperation of J.G. and E.G., the two study participants who were the focus of this investigation.

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1990, his performance was below expectation for estimated premorbid abilities in the domains of manual motor skills, attention, working memory/executive function, and visuospatial abilities. Assessment of short-term memory function showed deficits at the level of learning new information on several tasks, but his retention of newly learned information over delays was normal (i.e., he did not show significant forgetting of information over delays). In 1999, J.G.’s performance was within expected limits for estimated premorbid abilities in the domain of attention; however, he performed below expectation in the domains of motor function, working memory/executive function, and visuospatial functioning. On testing of short-term memory, performance was below expectation at the levels of both learning and retention of newly learned information (i.e., he showed significant forgetting). When comparing his 1999 performance with that in 1990, we used age-adjusted outcome measures to control for the age increase. His simple attention appeared to improve. The most dramatic decline was seen in the area of short-term memory, although his manual motor control was also somewhat worse. Scores on tasks assessing the domains of working memory/executive function and his drawings remained below expectation.

E.G. was tested only in 1999. Like his brother, he consistently did better on visuospatial than on verbal tasks, with verbal/language skills at the lower end of the average range and visuospatial skills at the upper end of the average range. Test performance was below expectation for estimated premorbid abilities in the domains of motor skills, working memory/executive function, and visuospatial abilities. On short-term memory tests, he performed somewhat below expectation at the level of learning on two tasks, but his retention of newly learned information over delays was normal.

A comparison of the 1999 assessments of each twin showed that both had mild manual motor deficits, but these appeared to be more pronounced in J.G. Scores on tests assessing working memory/executive function were likewise below expectation for both twins, although on slightly different tasks. Short-term memory function was dramatically worse in J.G. than in E.G., involving both the processes of learning and retention. The neuropsychological test results are shown in Table 1. Several test scores within each domain were judged by the neuropsychologist to be abnormal on the basis of estimated premorbid skills for each brother. The cognitive test results are consistent with frontal lobe dysfunction in both twins, but with rather dramatic hippocampal dysfunction in J.G.

**Bone lead levels and MRS.** In 1998, bone lead measurements were taken with a K-X-ray fluorescence (KXRF) bone lead analyzer (Aro et al. 1994; Chettle et al. 1991). We determined the ratio of N-acetylaspartate (NAA) to creatine, a marker of neuronal density, using MRS. From each brother we obtained 1.5-tesla single-voxel point resolved spectroscopy (PRESS) spectra [repetition time + echo time = 2,000/144 msec/msec with 128 averages] from five voxels. The voxel locations were in the left and right frontal lobes, the left and right hippocampi, and one voxel in the left midbrain encompassing the central semioval and selected from the same axial slice as the frontal lobe voxels. Voxel sizes were roughly 1.7 cm³. Spectral analysis was performed with software supplied by the manufacturer (SA/GE; General Electric Medical Systems, Milwaukie, WI) and consisted of spectral phasing followed by peak area fitting for the metabolite resonances of choline, creatine, and NAA. Independent spectral analyses were performed by two individuals with daily processing of clinical single voxel spectra, and the results from each region and operator were averaged. Examples of spectra from the left frontal lobes of each twin are shown in Figure 1. The bone lead concentrations and NAA:creatinine ratios from the MRS exams are summarized in Table 2. J.G. had much higher levels of trabecular (patella) lead and cortical (tibia) lead than did E.G. In general, J.G. demonstrated a decrease of 10–30% in the NAA:creatinine ratio compared with E.G.

**Discussion**
The case of these twins illustrates many of the classic clinical and public health issues continued, next page
surrounding acute and chronic adult lead toxicity and offers a unique opportunity to explore the utility of MRS for examining effects of lead exposure. Although both twins had elevated body burdens of lead, there were differences between them, which, in identical twins with very similar life exposures, provided an ideal opportunity to explore the use of MRS technology for assessing the impact of lead toxicity on the CNS and perhaps shed light on mechanisms of action.

**Lead exposure in Massachusetts.**

Construction work has become the dominant source of lead exposure for adults in the United States. In Massachusetts, 1 of the 27 states that currently maintain central registries of blood lead tests and report surveillance data to the National Institute for Occupational Health and Safety and Health Adult Blood Lead Epidemiology and Surveillance Program [Centers for Disease Control and Prevention (CDC) 1999], construction workers accounted for 63% of 381 individuals identified with PbP levels of ≥ 40 µg/dL—the action level in the Occupational Health and Safety Administration’s (OSHA) standard (Rabin et al. 1994). Most houses in the United States built before 1978 (estimated at 42–47 million houses) have lead-based paint inside and outside [Agency for Toxic Substances and Disease Registry (ATSDR) 1998]. Lead paint can contain up to 50% lead by weight, which poses an enormous risk to construction workers—including painters—who remove it as well as to children whose hand-to-mouth behavior and frequent floor activity raise their risk of ingesting lead paint chips and lead-contaminated house dust. Scraping, and, in particular, sanding lead paint creates a fine lead dust that can be easily inhaled. Absorption of lead is highly efficient after inhalation, particularly if the particles are small. Hand-to-mouth behavior of construction workers can also lead to significant absorption of lead, such as smoking cigarettes and eating without prior hand washing. Lead dust on the hands can be ingested and absorbed through the gastrointestinal tract as can lead dust on cigarettes, which can be heated during smoking generating lead fumes that are especially well absorbed by the lungs. In addition to use in residences, lead paint was also used in commercial buildings and other structures, such as bridges. Workers who remove paint in these sectors are at extremely high risk for lead exposure (Levin and Goldberg 2000). Construction work is regulated under the OSHA construction lead standard that took effect in 1993 (OSHA 1993), and some states have additional standards that apply specifically to the painting and deleading of residences. Such regulations require the use of certain personal protective equipment (e.g., special respirators) and work techniques that reduce exposure (e.g., “wet scraping” to reduce dust), as well as prohibit certain activities that increase exposure (e.g., smoking and eating at work). These regulations, however, are often difficult to enforce and do not apply to individual homeowners who undertake renovations themselves.

**Neurocognitive effects of lead.** Although lead has adverse effects on numerous health end points (ATSDR 1999), the most sensitive target of lead exposure is the nervous system. Neurologic functions for which there is evidence of an adverse effect of chronic exposure to lead include peripheral nerve conduction velocity, postural balance, visual and auditory evoked potentials, cardiac autonomic nervous system function, and neurocognitive functions mediated by the CNS (Araki et al. 2000;
The pattern of cognitive deficits in the twins that we report here is generally quite typical of the pattern of deficits reported after high-level lead exposure. This pattern includes predominant impairments in the domains of attention/executive function, visuospatial/visual motor functioning, short-term memory, and (for J.G.) confusion and fatigue, whereas verbal language and general intelligence remain relatively unimpaired. Test of single-word reading, basic written arithmetic, and semantic knowledge (e.g., the ability to name common objects) are not generally sensitive to exposure to neurotoxicants in adults. Disruptions of these types of cognitive functions are usually seen only after widespread brain damage (e.g., frank hypoxia, severe traumatic brain injury, Alzheimer disease after the initial stages) or focal strokes involving highly specific brain areas that mediate language and calculations. For these reasons, neuropsychologists often use these tests when evaluating adults with suspected CNS insults to estimate premorbid patterns and levels of cognitive function in different domains (especially verbal, visuospatial, and attention). After exposure to toxicants such as lead in adulthood, cognitive deficits tend to be specific, not generalized and not affecting language centers in the brain. In the case of lead, this is probably due to its action on hippocampal and frontal areas of the brain. In a recent study of cumulative (bone lead) exposure in a general population, Wright et al. (2003) found a significant association with slightly lower scores on the MMSE. Overall, J.G. scored lower on the neurocognitive testing than did his brother (E.G.), which is consistent with J.G.’s higher bone lead levels and lower NAA:creatinine ratios. In the case of the twins presented here, however, we cannot distinguish what effects might be related to high acute Pb concentrations as opposed to cumulative exposure reflected in the high bone lead levels. It should also be noted that other known neurotoxicants such as solvents are frequently used in painting. Some of the functional deficits noted in this study may in part be related to toxicants other than lead, and differential exposure to these other neurotoxicants could also contribute to some of the differences on cognitive tests between the twins.

The magnetic resonance images (MRIs) from the twins showed lesions indicative of microinfarcts. This is consistent with known adverse effects of lead on the cardiovascular system. In the context of the neurobehavioral deficits exhibited by the twins, it is possible that these outcomes are to some extent the result of adverse cerebrovascular events brought about as the result of chronic lead exposure. Such effects would constitute an indirect action of lead on neuronal density and neurobehavioral impairment through actions on the...
cerebrovascular system, in addition to the likely direct effects on the nervous system.

**MRS measurements of neuronal density.**

The effects of elevated blood and bone lead levels have been examined primarily in the context of behavioral and neuropsychologic evaluations. There has been a growing interest in the mechanisms by which lead disrupts brain function. Although the adverse effects of lead exposure on neurobehavioral functioning is one of the most consistently reported of lead exposure on neurobehavioral function—evaluations. There has been a growing interest likely direct effects on the nervous system. Such evaluation of the MRS pulse sequence used to acquire the data as the relaxation times, T_1 and T_2, because the different metabolites are not iden-

tical in control populations and populations in a number of MRS studies of brain metabo-
lites in control populations and populations with specific diseases such as amyotrophic lat-
eral sclerosis (ALS) and Alzheimer disease (Barker et al. 2000; Chan et al. 1999; Doraiswamy et al. 1998; Kreis et al. 1993; Lundbom et al. 1999). It is important to note that the NAA:creatine ratio has not only regional (Barker et al. 2000; Jayasundar and Raghunathan 1997; Kreis et al. 1993; Lundbom et al. 1999; Ricci et al. 2000) and developmental dependencies but also depends on the specific echo time and repetition time of the MRS pulse sequence used to acquire the data. Within the context of the present study, although there may not be an exact relation between the NAA:creatine ratios we obtained in the twin brothers and those found in other studies, the difference in NAA:creatine ratio between the twins spans a range on the order of that seen for elderly adults in other studies.

Although we cannot conclusively attribute the differences in NAA:creatine ratios between the brothers to the differences in lead exposure, the fact that these two brothers matched for genetics, education level, and many life experiences would support the hypothesis that the lower NAA:creatine ratios in J.G. are secondary to higher lead exposure. If so, this suggests that chronic lead exposure caused a loss of neu-
rons in the hippocampus, frontal cortex, and midbrain. Possible mechanisms of cell loss include lead-induced oxidative toxicity (Adonaylo and Oteiza 1999), cellular apoptosis without necrosis (Fox et al. 1998), and indirect oxidative toxicity via increases in the metabo-
late aminolevulinic acid (Bechara 1996). Clearly the study of the relation between lead exposure and neuronal density as assessed by MRS in a larger population will be necessary to determine these relations with more certainty.

**Conclusions**

Construction work has become the dominant source of lead exposure in U.S. adults. Neurobehavioral sequelae of lead toxicity are not uncommon and studies are beginning to suggest that these outcomes can occur with
chronic exposure at levels allowed under current U.S. regulation. Presenting symptoms of acute lead toxicity are often vague and may likely involve health end points other than neurobehavioral ones, such as the back pain that initially brought J.G.’s lead exposure to medical attention. Thus, particularly when dealing with construction workers, a high index of suspicion and a low threshold for testing BPb levels are called for in order to diagnose lead toxicity.

In the cases presented, both of the monozygotic twin painters clearly had extremely high bone lead levels. Nonetheless, their differential lead exposure resulting from different job tasks was reflected in differences in bone lead levels. On the background of genetic identity and extremely similar life exposures, the relations between lead levels, neuro-psychological testing, and MRS results are highly suggestive. The markedly higher bone lead levels in J.G. were paralleled by greater deficits in neuropsychological testing performance and lower NAA:creatine ratios in the hippocampus and frontal lobes. These results are consistent with neuronal loss secondary to lead exposure, which could be responsible in part for the impaired neuropsychological function on hippocampal and frontotemporal-dependent tasks. Although we cannot establish cause and effect, we believe that MRS may be a valuable research tool in determining the mechanisms of neurotoxicity of lead and potentially other neurotoxicants as well.

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