Magnetic Resonance Imaging and Spectroscopy of Regional Brain Structure in a 10-Year-Old Boy With Elevated Blood Lead Levels

ABSTRACT. *Objective.* **The effects of elevated blood lead levels on the development of children have been examined only in the context of behavioral and neuropsychological evaluations. No studies have examined the effects of lead on brain metabolism in vivo or on structural and/or functional correlates of brain function in children. In the human brain, magnetic resonance spectroscopy (MRS) provides a noninvasive, risk-free method to monitor the biochemistry of acute and chronic stages of disease. The purpose of this study was to examine in vivo the use of MRS for the evaluation of the neurotoxic effects of lead on the nervous system, by detection of brain metabolism, especially *N*-acetylaspartate, a metabolite shown to decrease in processes that involve neuronal loss.**

Methodology. **Two male cousins who live in the same household and share the same socioeconomic background and home environment were studied. The subject, a 10-year-old boy, had elevated blood lead levels. His cousin, a 9-year-old boy, was not exposed to lead. Both underwent a comprehensive neuropsychological evaluation and both were evaluated using the magnetic resonance imaging (MRI) and MRS at the University of Pennsylvania Medical Center. High-resolution MRI and MRS were performed using a 3-in surface coil. Localized proton spectra were obtained from contiguous 6 × 6 × 10-mm voxels using one-dimensional phase encoding, with a 2000-millisecond repetition time and a 31-millisecond echo time.**

Results. **Neuropsychological evaluation demonstrated areas of impairment in the lead-exposed child, including difficulties in academic skills of reading, writing, and arithmetic, as well as deficient linguistic skills and attentional mechanism. By contrast, studies of the cousin, who was not exposed to lead, showed overall neuropsychological functioning within normal limits. Although both children had a normal MRI examination of the brain, studies of the lead-exposed boy showed a significant alteration in brain metabolites, with a reduction in the *N*-acetylaspartate:creatine ratio for both gray and white matter on the MRS examination, compared with his cousin.**

Conclusions. **The present study is a first attempt to determine in vivo metabolic differences in the brain of a child exposed to lead compared with a healthy control subject. This is a unique case because these children were matched on a number of variables usually regarded as confounders in behavioral lead studies, and therefore can be regarded as matched controls. The present study demonstrates that MRS is a feasible, noninvasive technique for in vivo examination of the brains of children exposed to lead. We were able to obtain high-quality spectra from voxels as small as 0.36 cm at 1.5T. The spatial resolution used in the present study is sufficient to obtain spectra from voxels almost exclusively composed of gray matter. The one-dimensional phase-encoding approach used presents the advantage of obtaining several spectra simultaneously in a relatively short time. The present study has allowed us to examine the spectroscopic patterns of frontal gray and white matter after lead exposure relative to the normal pattern seen in healthy children and adults. The MRS study of the healthy, nonlead-exposed cousin demonstrated spectra entirely consistent with the spectral pattern reported in previous studies of healthy individuals. By contrast, the spectra obtained from the lead-exposed child deviated from the expected pattern in all metabolite ratios analyzed. Because *N*-acetylaspartate has been shown as a measure of neuronal viability, the level of *N*-acetylaspartate may enable us to evaluate the degree of neuronal loss in children exposed to lead. The MRI examination indicated no structural abnormalities or cortical thinning, and no abnormal findings in either case. By contrast, MRS indicated a significant change from normal values for the lead-exposed child. This supports the idea that lead neurodevelopmental toxicity may be related to interference with neurocellular development processes. The results are discussed in relation to the future use of MRS to detect metabolic abnormalities in children with lead poisoning. *Pediatrics* 1998;101(6). URL: http://www.pediatrics.org/cgi/content/full/101/6/e7; *lead, proton MRS, brain cortex, neuronal viability.***

ABBREVIATIONS. MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; NAA, *N*-acetylaspartate; CNS, central nervous system; Cho, choline-containing compounds; Cr, creatine and phosphocreatine; TR, repetition time; TE, echo time; VOI, voxel of interest; 1D, one-dimensional; mI, *myo*-inositol.

The effects of elevated blood lead levels on the development of children have been examined only in the context of behavioral and neuropsychological evaluations. Debate continues on the effects of low to moderate lead levels (10 to 40 μg/dL) on general cognitive functioning. One of the most consistently reported impairments associated with lead exposure at levels as low as 25 μg/dL involves its negative impact on general intellectual functioning.[1–6] There are no studies examining the effects of lead on brain metabolism in vivo or on structural and/or functional correlates of brain function in children. In the human brain, magnetic resonance spectroscopy (MRS) provides a noninvasive, risk-free method to monitor the biochemistry of acute and chronic stages of disease.[7–9] The development of spatial localization methods, which sample the relative levels of mobile metabolites from a volume of tissue defined from an MR image, has provided a basis for integrating the biochemical information obtained by MRS with the anatomic and

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pathologic information obtained from magnetic resonance imaging (MRI). This combination of metabolic and anatomic information affords a new means of understanding the origins and time course of progression in a variety of diseases. MRS has gained widespread acceptance as a method for assessing both neuronal viability as well as demyelination. This acceptance is based on the fact that one of the metabolites identified in proton spectra of the brain, *N*-acetylaspartate (NAA), is largely confined to neurons[10,11] and has been recognized as a neuronal marker. In brain cortex, NAA is located in neuronal cell bodies, whereas in white matter, it is located largely in axons. Therefore, a decrease in NAA can be considered an indicator of neuronal and axonal damage and loss.[12] Proton MRS has been used to study neurodegenerative processes, and a decrease of NAA has been a common finding in patients with Alzheimer's disease,[13,14] Parkinson's disease,[15] and Huntington's disease.[16] The decrease in NAA is measured relative to the level of creatine, a stable metabolite that shows practically no change after neuronal loss. Van der Knapp et al[17] demonstrated that increased cerebral atrophy was accompanied by lower ratios of NAA to creatine (Cr) in patients with demyelination disorders. Applying the technique to children, Kimura and colleagues[18] reported abnormally low NAA/Cr ratios in neurologically delayed infants compared with children with no known developmental delays, and Grodd and coworkers[19] reported a marked decrease of NAA in children with focal or generalized demyelination. Because there is evidence showing reduced NAA peaks in disease processes involving intellectual deterioration, it is reasonable to expect a decrease in NAA in the brain of children with clinical evidence of lead neurotoxicity.

We used MRS to examine a child, who had elevated blood lead levels, and his first cousin, who was not exposed to lead, to determine whether it might serve as a new technique for evaluating the effects of lead on the central nervous system (CNS). The technique, developed by Lopez-Villegas et al,[20] was used to obtain metabolic information differentially from gray and white matter using high spatial resolution proton MRS. They demonstrated that spectra from frontal gray matter showed choline-containing compounds (Cho)/Cr and NAA/Cr ratios significantly lower than those from white matter in healthy young adults. They also reported lower Cho and higher Cr content in gray matter. This method was used in the present study to compare spectroscopic values in a 10-year-old boy with elevated lead levels with values for his cousin, a healthy 9-year-old boy.

CASE REPORT

Two male cousins, MC and MM, live in the same household and therefore share the same socioeconomic background and home environment. They have been raised by the same parents (great-aunt and uncle) and have biological mothers who are sisters with similar home, socioeconomic, and educational backgrounds. MC was exposed to lead at the age of 24 to 48 months, when he spent time with his biological mother at his grandmother's home, although he continued to spend most of his time at his great-aunt's home, where lead was not present. MM tested negative for blood lead levels. The cousins were near the same age at the time of neuropsychological evaluation, although MC was ~1 year older than MM at the time of the MRI and MRS examinations. The only significant difference between the two children is lead exposure in MC.

MC

The patient, MC, is a 10-year-old, right-handed boy. He was born full-term after a normal pregnancy and birth. Developmental milestones occurred within normal time frames. He was first diagnosed with elevated blood lead levels when he was 38 months old, after a venous blood test. Documented blood lead levels ranged from 51 μg/dL at age 38 months to 44 μg/dL at 41 months. MC's schooling began in preschool at age 3 years. There were no difficulties reported until he was in second grade. His teachers found that he was slow to learn, and he repeated the second grade. At the time of his evaluation, he was in the third grade. His teacher reported reading, writing, and arithmetic skills below grade level, but grade-appropriate achievement in social studies and science. The neuropsychological evaluation indicated full scale IQ of 90, with verbal IQ of 95 and performance IQ of 84, with considerable intersubtest variability indicating uneven application of intellectual abilities (*Wechsler Intelligence Scale for Children, 3rd Edition*).[21] Low scores on digit span, arithmetic, and picture completion tests reflected impairments in attention and mental control. Reading, spelling, and arithmetic calculations presented as areas of difficulty for MC, with scores on the Woodcock–Johnson Tests[22] within the borderline range and reflecting inappropriate school learning. Reading was nonfluent, and MC experienced difficulties in reading at first-grade-level complexity (Letter–Word Identification) and demonstrated deficient phonics skills (Word Attack). He also experienced difficulties in spelling. Although he was able to count and perform simple applied arithmetic operations such as adding coins, MC showed significant impairment in performing two-digit subtractions and single-digit multiplication. In contrast, his scores on measures of general knowledge were significantly higher and within the average to the high average range. Semantic verbal fluency as measured by the Animal Naming Test[23] was within normal limits for animals (73rd percentile) but significantly below average for foods (6th percentile), as was word fluency.[24] On the Wide Range Assessment of Memory and Learning,[25] MC demonstrated difficulties in rote, sequential short-term memory for both verbal (Number–Letter Memory) and spatial material (Finger Windows). His performance improved somewhat when presented with meaningfully organized verbal material (Story Memory) and repeated presentations of verbal material (Verbal Learning). MC's general visual–motor integration, as measured by the VMI,[26] was within normal limits (standard score, 95; 37th percentile), as was his score on the Draw-A-Man test,[27] (standard score, 98; 45th percentile). However, his performance was impaired on the Purdue Peg board Test[28] when working with his right, dominant hand, with a score below the 10th percentile for his age. He performed within normal limits with his left hand.

MM

A 9-year-old ambidextrous boy, MM was born full-term after a normal pregnancy and birth. Developmental milestones were attained within a normal time frame, and there was no history of developmental or school difficulties.

On neuropsychological evaluation, in contrast to MC's variable performance, MM's intellectual ability was within the high average range (full scale IQ, 112; verbal IQ, 111; and performance IQ, 112), with rather even performance and no outstanding strengths or weaknesses. Performance on the Woodcock–Johnson Tests was generally within the average to high average range, indicating age-appropriate school learning. He demonstrated particularly developed phonics skills, as evidenced by superior performance on Word Attack. His performance on all measures of language was within normal limits. He demonstrated well-developed reading and writing skills and above average semantic and word-fluency skills. MM's overall performance on the Wide Range Assessment of Memory and Learning was within the average range. However, he showed particular weakness when asked to repeat meaningful sentences verbatim; this represented the only limitation in his profile. MM's performance on visual–motor integration was well above average (standard score, 122; 93rd percen-

tile). His performance on the Draw-A-Man test also was above average (standard score, 114; 82nd percentile). Pure motor dexterity, assessed by the Purdue Pegboard Test, was above average for his left, dominant hand (60th percentile) and both hands working simultaneously (90th percentile), but somewhat below average with his right hand (20th percentile).

All MR studies were performed at the Hospital of the University of Pennsylvania in Philadelphia, PA, on a 1.5T Signa system (GE Medical Systems, Milwaukee, WI). Conventional MRI was performed with a standard quadrature head coil, which was then replaced with a 3-inch surface coil positioned over the left frontal region immediately supraciliary. A sagittal localizer was obtained, followed by axial three-dimensional spoiled Gradient Recalled Acquisition in the Steady State [GRASS] (3D-SPGR) images (256 × 256 matrix; 8-cm field of view; 22.4 millisecond repetition time (TR); 7.5 millisecond echo time (TE); 45° flip angle; two acquisitions; 1.5-mm thickness; 28 sections). The 3D-SPGR images provide high contrast between gray and white matter and were used to choose the voxel of interest (VOI) for the spectroscopic study. Immediately after high-resolution MRI, one-dimensional (1D) proton spectra were obtained with the stimulated-echo acquisition mode for localization. Water suppression was achieved by using three chemical shift-selective radio frequency pulses, followed by a dephasing gradient applied on each of the three axes. The sequence parameters included the following: 19-cm field of view; 2500-Hz spectral bandwidth; 32 phase-encoding steps; 2000-millisecond TR; 31 millisecond TE; 10.6-millisecond mixing time; 2048 complex points, eight-step phase cycling, and 16 acquisitions. We selected a VOI of 30 to 40 × 6 × 10-mm, including cortical gray and white matter. Spectra from contiguous 6 × 6 × 10-mm voxels were obtained from the VOI by 1D phase-encoding. Cortical sulci were included in the VOI in all cases. Because the thickness of cortical gray matter is ~3 mm,[29] the inclusion of cortical sulci in the VOI guarantees an approximate 6-mm thickness of gray matter. To avoid partial volume effects, the spatial distribution of gray and white matter included in the VOI had been checked to be relatively invariant in at least six of the MR images (1.5-mm contiguous sections) that contributed to the MRS section (10-mm thickness). Scalp and marrow were excluded from the VOI to prevent contamination from lipids. Gradient shimming on the VOI and optimization of solvent suppression were performed before the start of the acquisition. The spectral acquisition time was 17 minutes, and the total examination time, including MRI and MRS studies, was ~55 minutes. The MR procedure was well tolerated by both patients.

The spectral processing was performed with ProNMR (Softpulse Software, Guelph, Ontario, Canada) using zero filling to 4K data points, 1.5 Hz line broadening applied in the time domain, two-dimensional Fourier transformation, and zero-order phase. Areas under the peaks were determined using a Marquardt fitting routine to Lorentzian line shapes in the frequency domain (ProNMR), and peak area ratios were calculated. MRI and MRS studies were evaluated blind to the status of the patients.

A representative study showing the VOI prescription in the left prefrontal lobe, along with the stack-plot of proton spectra from adjacent voxels obtained by 1D phase-encoding, is shown in Fig 1.

The signal-to-noise ratio from spectra coming from the margins of the VOI was lower compared with intermediate voxels, probably because of partial volume effects. Typical spectra from frontal gray matter and white matter with the principal metabolites identified are shown in Fig 2 for MC (A) and MM (B).

The peak assignments were based on the published literature, and the chemical shifts were determined using NAA as a chemical shift standard. The following resonances were assigned: NAA (2.0 ppm, 2.6 ppm); Cr (3.0 ppm, 3.9 ppm); Cho (3.2 ppm); and *myo*-inositol (mI) (3.5 ppm). The region between 2.1 and 2.5 ppm contains peaks from glutamate, glutamine, gamma-amino butyric acid, and NAA. These peaks could not be resolved because of the overlap of resonances. Other peaks from glutamate and glutamine are contained in the region between 3.6 and 3.8 ppm. Residual lipid signals were identified in the region between 0.5 and 1.5 ppm. The peaks at 2.01 ppm and 3.0 ppm were used for the quantification of NAA and Cr, respectively.

The results of an analysis of peak area ratios for gray and white matter are summarized in Table 1 for the two patients.

DISCUSSION

The present study is a first attempt to determine in vivo metabolic differences in the brain of a child exposed to lead compared with a healthy control subject. This is a unique case because these children were matched on a number of variables usually regarded as confounders in behavioral lead studies and therefore can be regarded as matched controls. Neuropsychological evaluation demonstrated areas of impairment in MC, consistent with reports in the literature describing the detrimental effects of lead on the cognitive and behavioral development of children.[1–6] More specifically, the difficulties in academic skills of reading, writing, and arithmetic as well as the deficient linguistic skills and attentional mechanism seen in MC all have been associated with lead exposure.[6,30–32] By contrast, MM's overall cognitive and neuropsychological functioning was within normal limits. Although neuropsychological evidence is of great importance in determining the cognitive and behavioral sequelae of lead exposure, it does not provide insight as to the mechanisms by which lead affects brain substrate. The current study provided a first, albeit preliminary, insight to the direct effect lead has on brain metabolites by showing spectral abnormalities after exposure to lead.

The MRS study of MM, the healthy, nonlead-exposed cousin, resulted in spectra entirely consistent with the spectral pattern reported in previous studies for healthy individuals[20,33,34] documenting the

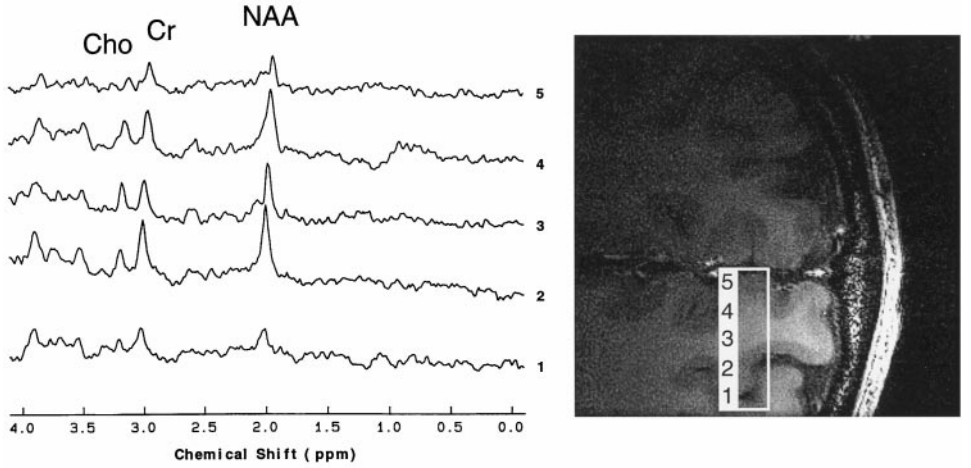

Fig 1. A representative study showing the VOI prescription in the left prefrontal lobe obtained in a healthy subject along with the stack-plot of proton spectra from adjacent voxels obtained by 1D phase-encoding.

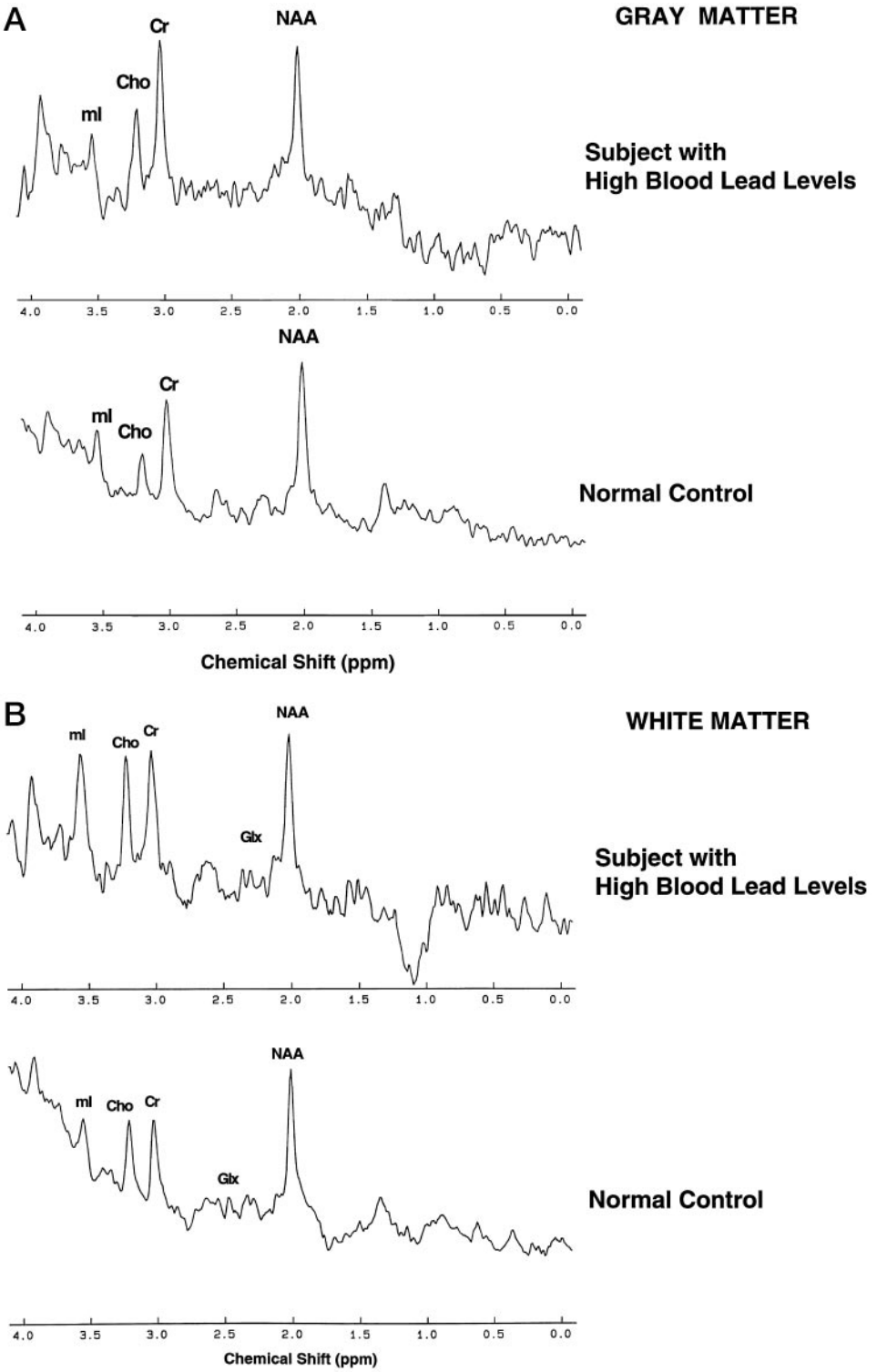

Fig 2. A, Spectra from frontal gray matter, with the principal metabolites identified for the subject with high blood lead levels (MC) and for a healthy control subject (MM). B, Spectra from frontal white matter, with the principal metabolites identified for the subject with high blood lead levels (MC) and for a healthy control subject (MM).

levels of these metabolites in the healthy adult brain as well as the estimated metabolite concentrations. These studies demonstrated that spectra from frontal gray matter are characterized by lower Cho/Cr and NAA/Cr ratios compared with ratios obtained from white matter. Using the same technique as that used in the present study, Lopez-Villegas and colleagues[20] also reported that in healthy young adults, there were no differences in mI/Cr ratios for gray and white matter. The spectra obtained from MM showed the same pattern of metabolite ratios. Although in the immature brain, NAA is present in immature oligodendrocytes as well as in neurons, we believe that it is appropriate to compare the spectra obtained from MM with those obtained in the Lopez-Villegas study, because the level of NAA/Cr has been reported to become constant at ~3 years of age.[18]

In contrast to the spectra obtained from MM, the spectra obtained from MC, the lead-exposed child, deviated from this expected pattern in all metabolite ratios analyzed. The NAA/Cr ratio was substantially

TABLE 1. Metabolite Ratios for MC and MM*

		MC	MM
NAA/Cr	GM	0.83 ± 0.16	1.29 ± 0.08
	WM	1.03 ± 0.11	1.59 ± 0.08
Cho/Cr	GM	0.45 ± 0.04	0.44 ± 0.04
	WM	0.50 ± 0.03	0.61 ± 0.17
mI/Cr	GM	0.22 ± 0.06	0.61 ± 0.17
	WM	0.40 ± 0.007	0.57 ± 0.007

* Numbers represent average spectra.
GM indicates gray matter; WM, white matter; NAA/Cr, *N*-acetylaspartate/creatine; Cho/Cr, choline/creatine; mI/Cr, *myo*-inositol/creatine.

lower for both gray and white matter in MC compared with MM (Table 1). Previous studies have linked lowered NAA/Cr ratios to neuronal loss and decline in intellectual functioning.[10,13,17,18] Therefore, the lowered NAA/Cr ratio in MC is suggestive of significant neuronal loss in the region examined. There is no indication in MC's developmental history of any event other than lead exposure that would result in loss of brain neurons. Therefore, the reduction in NAA/Cr ratio may be a direct result of his elevated lead levels.

We have found a significant decrease in the mI/Cr ratio of MC, the child exposed to lead. The mI peak consists mainly of mI (70%) but also contains mI-monophosphate (15%) and glycine (15%).[35] Although the role of mI in the CNS is still not understood completely, the level of mI has been found to be increased in CNS diseases such as Alzheimer's disease,[36] diabetes mellitus,[33] and metachromatic leukodystrophy,[37] and decreased in patients with chronic hepatic encephalopathy.[38] Shonk and Ross[39] have reported increased mI/Cr ratios in Down's syndrome. mI is the precursor of inositol-phosphates, which are important secondary messengers involved in a large number of hormonal systems and enzyme regulation in the CNS. mI may act as an organic osmolyte. It is an essential growth factor[35] and the precursor of phosphatidylinositol, a constituent of phospholipid membranes.[38] In the brain, mI has been suggested as a glia-specific marker for in vivo nuclear MR studies[40] because it is located primarily in glial cells and not in neurons.

Another interesting finding of the present study was the results of the MRI examination, which indicated no structural abnormalities or cortical thinning, and no abnormal findings in either case. By contrast, MRS indicated a significant change from normal values for MC but not for MM. This supports the idea that lead neurodevelopmental toxicity may be related to interference with neurocellular development processes. Thus, MRS may prove to be a more sensitive technique for the detection of brain abnormalities than is MRI.

It is encouraging also that in these matched children, the neuropsychological deficits and MRS abnormalities were in agreement for the lead-exposed boy, in that both cognitive–behavioral measures and spectra analysis demonstrated significant abnormalities after lead exposure. Although it is not possible at this early stage or based on two cases to link the neuropsychological findings with the MRS spectra, MRS holds promise for establishing such a link and enabling a more detailed evaluation of specific regions that might be more sensitive to the effect of lead. Additional research using MRS is underway in our center to determine more precisely what effect lead might have on the developing nervous system.

In summary, the present study demonstrates that MRS is a feasible, noninvasive technique for in vivo examination of the brains of children exposed to lead. The children were not sedated and participated willingly. This study demonstrates that MRS can be used as a technique to measure brain metabolites in vivo. Because NAA has been shown as a measure of neuronal viability,[10–12] the level of NAA may enable us to evaluate the degree of neuronal loss in children exposed to lead. We were able to obtain high-quality spectra from voxels as small as 0.36 cm at 1.5T. The spatial resolution used in the present study is sufficient to obtain spectra from voxels almost exclusively comprising gray matter. The 1D phase-encoding approach used presents the advantage of obtaining several spectra simultaneously in a relatively short time. The present study has allowed us to examine the spectroscopic patterns of frontal gray and white matter after lead exposure relative to the normal pattern seen in healthy children and adults.

Although we have demonstrated differences in metabolites in regions in the frontal lobe, additional studies confirming these differences as well as sampling different regions in the brain will be helpful in establishing whether lead affects specific brain regions or, alternatively, whether it affects the brain more diffusely. The potential for this technique in determining the specific effect of lead on the CNS appears feasible and significant. This technique presents opportunities for the investigation of the brains of children and adults with lead poisoning to determine more precisely the effects of lead on the brain and to examine any regional metabolic abnormalities.

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