

Altered neurometabolite development in HIV-infected children

Correlation with neuropsychological tests

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Abstract—Background: HIV-infected children have abnormal cerebral metabolites, measured by proton MR spectroscopy ($^1\text{H-MRS}$), but how these abnormalities relate to brain function is unclear. **Methods:** Metabolite concentrations in five brain regions of 20 HIV-infected and 13 control children were measured, and these findings were correlated with age, \log_{10} plasma viral load, CD4 count, and neuropsychological scores. **Results:** Compared with control subjects, HIV patients had decreased choline concentration [Cho] in left frontal white matter (LFW) (-12% ; $p = 0.04$); those with high viral load ($>5,000$ HIV RNA copies/mL) had decreased right basal ganglia (RBG) [Cho] (-15% ; $p = 0.005$), and [Cr] (-13% ; $p = 0.02$). Patients with high viral load also had higher [Cho] in the midfrontal gray matter (MFG) ($+25\%$; $p = 0.002$) and lower myo-inositol [Ins] in the RBG (-18% ; $p = 0.04$) than patients with low HIV viral load. *N*-Acetyl aspartate concentration ([NAA]) correlated with age in right frontal white matter (RFW) ($r = 0.59$, $p = 0.04$), LFW ($r = 0.66$, $p = 0.02$), and right hippocampus (RHIP) ($r = 0.69$, $p = 0.02$) only in control subjects. In contrast, [Ins] correlated with age in both RFW and LFW ($r = 0.71$, $p = 0.0006$; $r = 0.65$, $p = 0.006$) only in the HIV patients. \log_{10} plasma viral load correlated positively with [Ins] in RFW ($r = 0.54$, $p = 0.02$) and [Cho] in MFG ($r = 0.49$, $p = 0.04$). Compared with control subjects, HIV patients had poorer spatial memory ($p = 0.045$) and delayed spatial memory correlated with [Cho] in RHIP ($r = 0.68$, $p = 0.02$). **Conclusions:** These data suggest that normal brain development may be affected in children infected with HIV at birth, particularly evidenced by the lack of age-related increases in the neuronal marker [NAA]. Early, aggressive treatment of infants with HIV before development of encephalopathy is warranted.

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Proton MR spectroscopy ($^1\text{H-MRS}$) provides a noninvasive technique for measuring cerebral metabolites that reflect the metabolic integrity and density of neurons and glial cells. The most commonly studied metabolites have been *N*-acetyl aspartate (NAA), a neuronal marker, choline (Cho), which assesses membrane turnover, myelination or myelin breakdown, and gliosis, myo-inositol (Ins), a glial marker, and total creatine (Cr), which reflects the energy metabolism.^{1–3} Most MRS studies of HIV have relied on ratios of metabolites [NAA], [Cho], and [Ins] to total creatine [Cr], the sum of creatine and phosphocreatine, although some investigators have reported metabolite concentrations in absolute or arbitrary units.^{4–6}

Metabolite abnormalities assessed by $^1\text{H-MRS}$ have been reported in both white and gray matter of

adult HIV patients with dementia or less severe cognitive and motor impairment,^{4–14} particularly decreased [NAA] or [NAA]/[Cr], increased [Cho] or [Cho]/[Cr], and increased [Ins] or [Ins]/[Cr]. Metabolite abnormalities also correlated with the severity of neurologic impairment^{4,13,15,16} and may improve with antiretroviral therapy.^{12,17} Elevated Cho with normal [NAA] can be detected in subcortical areas, including frontal white matter¹⁵ and thalamus⁵ and the gray matter of asymptomatic HIV patients.^{8,15} However, others observed decreased [NAA]/[Cr] in the thalamus and centrum semiovale of asymptomatic HIV patients.¹⁰ A recent study also found correlations between frontal lobe [Ins] with cognitive performance on neuropsychological tests.¹⁵

Studies in HIV-infected children have been fewer but have confirmed some of the findings in adults. Earlier studies¹⁸ found decreased [NAA] and increased [Ins] relative to the sum of multiple other metabolites in the white matter (centrum semiovale) of children with encephalopathy. Decreased [NAA]/

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[Cr] also was reported in basal ganglia of pediatric patients with HIV encephalopathy.^{19,20} Another large study of 45 children with AIDS²¹ also examined the basal ganglia and found decreased [NAA]/[Cr] for patients with encephalopathy and decreased [Cho]/[Cr] for pediatric HIV patients without encephalopathy compared with control children. None of these prior studies evaluated the relationship between cognitive function and brain metabolite changes in HIV-infected children.

To further advance these prior studies, we assessed both absolute metabolite concentrations and metabolite/Cr ratios and used short echo time (TE) ¹H-MRS to measure [Ins], a marker of glial proliferation. Our study also evaluated five different brain regions in each of the HIV-infected and control children. In addition, we determined correlations between metabolites and patient characteristics such as age, viral load, CD4 counts, and performance on a panel of neuropsychological tests.

Methods. Subjects. Twenty HIV-infected children and 13 control children, ages 6 to 16 years, were enrolled in the study. The protocol and consent forms were approved by the Institutional Review Board at Harbor-UCLA Research and Education Institute. Assent was also obtained for children over age 8. Entry criteria for all subjects were 1) ages 6 to 16 years and 2) informed consent to participate in the study from a parent or legal guardian and assent from children over age 8. HIV subjects additionally required confirmed diagnosis of HIV-1. Exclusion criteria for the HIV patients were 1) opportunistic infections of the CNS, 2) other CNS diseases (e.g., brain neoplasms, multiple sclerosis), 3) severe metabolic disturbances (e.g., renal or hepatic failure), and 4) metallic implants that would be hazardous for MR studies. For control subjects, the exclusion criteria additionally included 1) chronic medication, 2) neuropsychological impairment, and 3) severe school difficulties. Subjects were not sedated for the MR scans. CD4 percent (CD%), CD4 count (no. of cells/mm³), viral load (no. of HIV RNA copies/mL), and log₁₀ viral load were determined as part of the routine clinical care within 8 weeks of MRS date. The Los Angeles Pediatric Spectrum of Diseases (HIV Surveillance) Study assisted with historical data retrieval.

MRI and ¹H-MRS. The MRI protocol consisted of the following sequences: 1) a sagittal T1-weighted localizer (repetition time [TR]/TE = 300/8 milliseconds, 5-mm slice thickness, 2.5-mm gap, 22-cm field of view), 2) coronal fast spin echo images (TR/TE1/TE2 = 3,000/100/8, 5.0-mm slice thickness, 1-mm gap, and 24-cm field of view), and 3) axial inversion recovery (IR) MRI (TR/TE = 4,500/28 milliseconds, IR = 1,200 milliseconds, 4-mm slice thickness, no gap, 24-cm field of view). The spectroscopic locations were prescribed using coronal images for the right hippocampus (RHIP) and axial images for the other regions.

Water-suppressed ¹H MR spectra were acquired from five different locations, namely, right frontal white matter (RFW), left frontal white matter (LFW), midfrontal gray matter (MFG), right basal ganglia (RBG), and right hippocampus (RHIP). The water suppression was achieved by a CHESS sequence²² and volume localization with PRESS sequence.²³ Numerically optimized Shinnar-Le Roux slice-selective radiofrequency pulses²⁴ were used for PRESS (90, 180, 180°). The CHESS sequence consisted of three frequency-selective 90° pulses, each followed by dephasing B₀ gradient pulses.

The following parameters were used for the spectral acquisition: TR = 3,000 milliseconds, TE = 30 milliseconds, and 64 excitations. Eight unsuppressed water signals were acquired from each location for eddy current compensation and phase correction. A 1.5 T GE Signa MRI/MRS scanner (GE Medical Systems, Milwaukee, WI) operating in the 8.X mode with "echo speed" gradients (23 mT/m) was used. A standard birdcage quadrature MRI head coil was used. The MRS protocol consisted of acquisition of metabolite and water signals after optimization of 90/180 radiofrequency pulses, automated shimming using the localized water

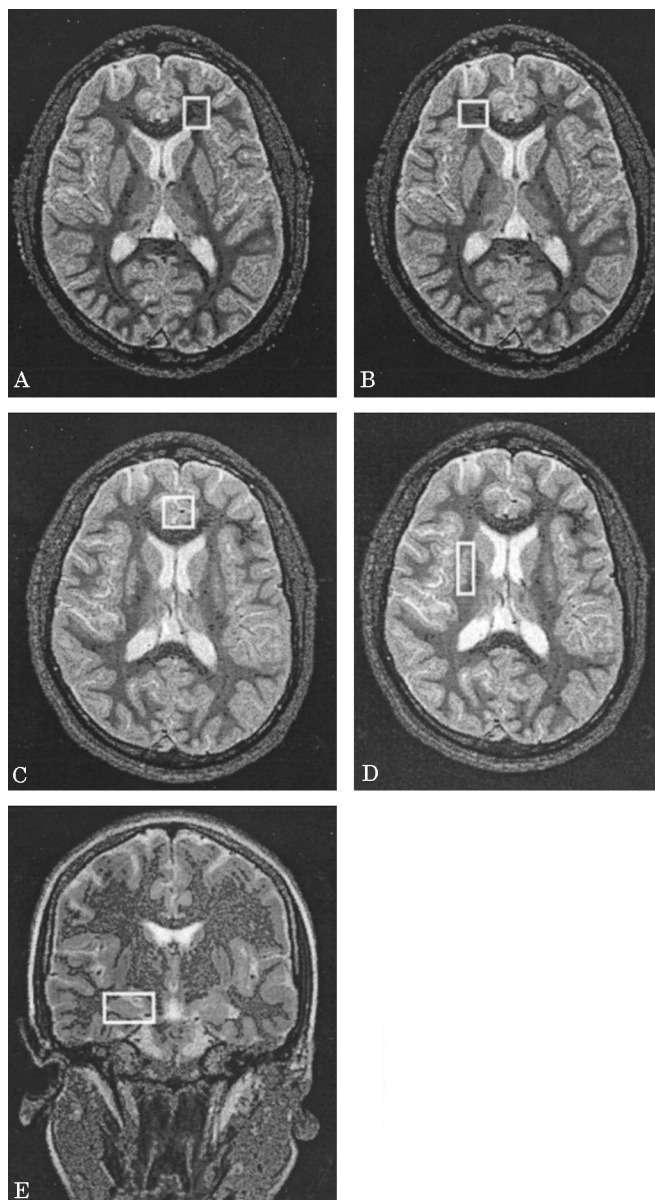


Figure 1. Voxel locations for proton MR spectroscopy. (A) Left frontal white matter; (B) right frontal white matter; (C) midfrontal gray matter; (D) right basal ganglia; (E) right hippocampus.

signal, and water suppression. The voxel sizes for different locations were approximately 2 mL but varied with the location of the voxel and the size of the head. Voxel locations are shown in figure 1.

The MR data were transferred to an SGI workstation (Silicon Graphics, Santa Clara, CA) and processed using the LC-Model²⁵ package. Spectra were discarded if the signal-to-noise ratio was <3, and metabolite concentrations were discarded if the percentage standard deviation was >30%. Only spectra with a full width at half-maximum of ≤6.5 Hz are reported. The MR visible water concentration was analyzed with the LC-Model (white matter: 35,880 mM; gray matter: 43,300 mM). The absolute concentrations of different metabolites were obtained without correcting for T2 and T1 saturation or atrophy. The ratios were calculated with respect to [Cr]. Sample spectra from patients and controls are shown in figure 2.

Neuropsychological assessments. A panel of tests was performed, and scores were normalized for age. Testing was conducted primarily in English, with instructions given in Spanish, if necessary. Tests used were as follows: Block Design (a subtest of the Wechsler Intelligence Scale for Children, 3rd ed.); Children's

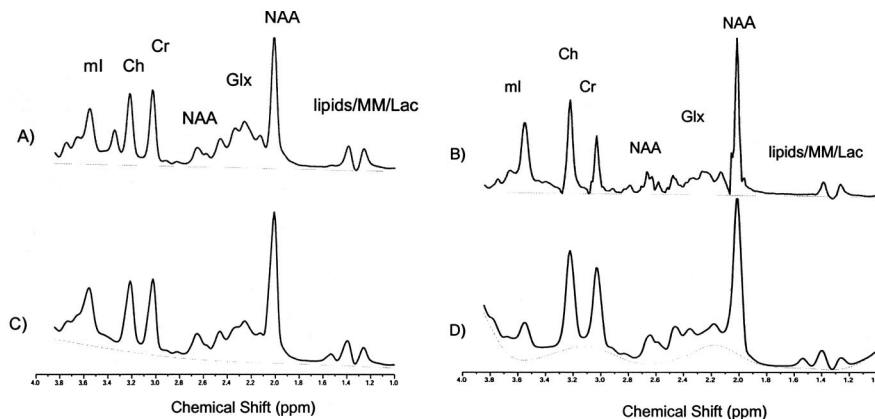


Figure 2. Spectra from a 15-year-old patient (A and B) and two different control subjects 13 years (C) and 16 years (D) of age. Spectra on left (A and C) are from right frontal white matter and spectra on right (B and D) are from left frontal white matter. mI = myo-inositol; Ch = choline; Cr = creatine; NAA = N-acetyl aspartate; GLX = glutamate and glutamine; MM = macromolecules; Lac = lactate.

Depression Inventory Total (CDI; a self-report of depressive symptoms); Children's Memory Scale (CMS; a spatial memory test); CMS Long Delay (spatial memory after long delay); Expressive One Word Picture Vocabulary Test, rev. (EOWPVT-R; a naming task); California Verbal Learning Test for Children, Short and Long Delay (a test of verbal memory of a list of words); Peabody Picture Vocabulary Test, rev. (a test of comprehension vocabulary and auditory receptive language); Test de Vocabulario en Imágenes Peabody (Spanish version); Purdue Pegboard Dominant and Nondominant Hands (tests of motor function); and Visual Motor Integration Test (Beery Test of Visual Motor Integration; copying geometric figures). Results were converted to standard scores with a mean of 100 and an SD of 15 with the exception of Block Design, CMS Total, and CMS Long Delay, which are presented as scaled scores (mean = 10, SD = 3).

Statistics. Comparisons of patient and control subjects on metabolite concentrations and metabolite/[Cr] ratios and neuropsychological tests were performed using *t*-tests. Pearson correlations were used to test the association of metabolite concentrations and metabolite/[Cr] ratios with age at time of study, age at treatment for HIV, CD4 count, viral load, log viral load, and neuropsychological assessments. The correlations were performed separately for patients and control subjects.

The Fisher *z* transformation (inverse hyperbolic tangent) was used to compare correlations on the same pairs of variables in patients and control subjects. In separate analyses, a subgroup of patients with higher viral load (>5,000 HIV RNA copies/mL) was compared with the control subjects and the patients with lower viral load (<5,000 HIV RNA copies/mL) on the metabolite concentrations and metabolite/[Cr] ratios. A type 1 error of 0.05 was used for all tests; correction for multiple comparisons was not performed. We also examined the linear regression data visually to determine if significant correlations reflected a consistent pattern of association. Linear regression data dominated by outlier points were excluded from further analysis.

Results. Subjects. Patient characteristics are provided in table E-1 on the *Neurology* Web site (go to www.neurology.org). The mean \pm SD age of HIV-infected children was 10.6 ± 3.3 years and of control children 10.7 ± 2.9 years. Of the HIV-infected children, 19 acquired their infection by vertical transmission from the mother, whereas 1 patient acquired infection through a blood transfusion at age 16 months. Fourteen patients had an HIV viral load of <5,000 HIV RNA copies/mL. All patients were receiving antiretroviral therapy: Nineteen patients were receiving highly active antiretroviral regimens (HAART), and one patient was receiving combination therapy with two antiretroviral drugs. Only three patients had received HAART for <1.5 years at the time of study. Maternal substance abuse history was available for 12 children. Three of these 12 were exposed in utero to maternal substance abuse.

Eight of the 20 HIV-infected patients were known to have a history of abnormal neurodevelopmental testing,

developmental delay, or significant school difficulties. Two of these eight children had been previously diagnosed with HIV encephalopathy (one child based on developmental delay and the other child based on psychiatric illness and developmental delay). This second child also had been exposed in utero to heroin and cocaine. Of the remaining six children, two had poor school performance and one had resolved developmental delay. Three of the six children had received psychiatric/psychological care. Only two of these eight children had been treated for HIV in the first year of life.

Structural MRI findings. Fifteen of the 20 HIV-infected patients had a normal MRI of the brain. Mild abnormalities were noted in the remaining five patients: Two had prominence of the perivascular spaces in the frontal lobes; one patient had punctate focal areas of abnormally increased signal predominantly in the subcortical white matter of the frontal lobes and the area around the atria of the lateral ventricles; another patient had a hazy abnormal signal in the periventricular white matter; and one patient had atrophy described as mild atrophy of the cerebellum vermis with slight prominence of the fourth ventricle.

Metabolite concentrations and metabolite/Cr ratios. A summary of the cerebral metabolite concentrations and metabolite/Cr ratios for each brain region in patients and control subjects is presented in table 1. Metabolite concentrations and metabolite/Cr ratios were similar for HIV-infected children and control subjects with only one exception: [Cho] in the LFW was 12% lower ($p = 0.04$) in the HIV-infected children (1.46 ± 0.21 mM) than control subjects (1.65 ± 0.25 mM).

All metabolites and metabolite/Cr ratios in each of the five brain regions were grouped by patients' HIV viral load into high viral load (>5,000 copies/mL) and low viral load (<5,000 copies/mL). Six patients with high HIV viral load had lower [Cho] (-15% ; $p = 0.005$) in the RBG compared with controls (0.97 ± 0.07 vs 1.14 ± 0.17 mM) but higher [Cho] in the MFG ($+25\%$; $p = 0.002$) compared with patients with low HIV viral load (1.30 ± 0.12 vs 1.04 ± 0.15 mM). [Cr] in the RBG was lower for patients with high viral load (-13% ; $p = 0.02$) than control patients (5.63 ± 0.49 vs 6.44 ± 0.71 mM). [Ins] in the RBG was 18% lower ($p = 0.04$) for patients with high HIV viral load than patients with low viral load (2.63 ± 0.37 vs 3.22 ± 0.47 mM).

Patients with an abnormal MRI of the brain (five patients) were compared with control subjects for each of the

Table 1 Comparison of metabolite concentrations (mM) and metabolite ratios from ¹H-MRS

Parameter	NAA	Cr	Cho	Ins	NAA/Cr	Cho/Cr	Ins/Cr
Right frontal white matter							
HIV patients	6.59 ± 0.91 (n = 19)	4.12 ± 0.57 (n = 19)	1.45 ± 0.23 (n = 19)	4.23 ± 1.23 (n = 19)	1.61 ± 0.21 (n = 19)	0.36 ± 0.07 (n = 19)	1.03 ± 0.27 (n = 19)
Controls	7.05 ± 1.21 (n = 12)	3.95 ± 0.55 (n = 12)	1.49 ± 0.30 (n = 12)	3.58 ± 1.31 (n = 9)	1.81 ± 0.31 (n = 12)	0.38 ± 0.08 (n = 12)	0.91 ± 0.42 (n = 9)
<i>p</i> Value	0.24	0.41	0.66	0.21	0.05	0.34	0.35
Left frontal white matter							
HIV patients	6.52 ± 0.64 (n = 17)	4.11 ± 0.86 (n = 17)	1.46 ± 0.21 (n = 17)	4.54 ± 1.62 (n = 16)	1.65 ± 0.34 (n = 17)	0.37 ± 0.10 (n = 17)	1.13 ± 0.38 (n = 16)
Controls	6.64 ± 0.59 (n = 12)	3.96 ± 0.49 (n = 11)	1.65 ± 0.25 (n = 12)	4.10 ± 1.26 (n = 11)	1.71 ± 0.24 (n = 11)	0.42 ± 0.06 (n = 11)	1.08 ± 0.48 (n = 10)
<i>p</i> Value	0.60	0.60	0.04*	0.46	0.61	0.14	0.75
Midfrontal gray matter							
HIV patients	7.19 ± 0.82 (n = 18)	5.42 ± 0.70 (n = 18)	1.13 ± 0.19 (n = 18)	5.02 ± 0.64 (n = 18)	1.34 ± 0.17 (n = 18)	0.21 ± 0.04 (n = 18)	0.94 ± 0.18 (n = 18)
Controls	7.56 ± 0.71 (n = 13)	5.52 ± 0.51 (n = 13)	1.23 ± 0.15 (n = 12)	4.82 ± 1.07 (n = 12)	1.38 ± 0.17 (n = 13)	0.22 ± 0.03 (n = 12)	0.86 ± 0.16 (n = 12)
<i>p</i> Value	0.20	0.66	0.13	0.53	0.52	0.43	0.21
Right basal ganglia							
HIV patients	6.83 ± 1.12 (n = 18)	6.08 ± 0.94 (n = 18)	1.05 ± 0.18 (n = 18)	3.06 ± 0.51 (n = 15)	1.14 ± 0.19 (n = 18)	0.18 ± 0.03 (n = 18)	0.51 ± 0.09 (n = 15)
Controls	6.69 ± 0.68 (n = 13)	6.44 ± 0.71 (n = 13)	1.14 ± 0.17 (n = 13)	3.10 ± 0.63 (n = 10)	1.05 ± 0.16 (n = 13)	0.18 ± 0.03 (n = 13)	0.47 ± 0.07 (n = 10)
<i>p</i> Value	0.69	0.26	0.17	0.87	0.20	0.80	0.25
Right hippocampus							
HIV patients	5.16 ± 0.73 (n = 13)	4.79 ± 0.64 (n = 13)	1.32 ± 0.21 (n = 13)	4.78 ± 0.63 (n = 13)	1.09 ± 0.19 (n = 13)	0.28 ± 0.07 (n = 13)	1.01 ± 0.16 (n = 13)
Controls	4.93 ± 0.37 (n = 11)	4.82 ± 0.35 (n = 11)	1.31 ± 0.21 (n = 11)	4.39 ± 0.87 (n = 11)	1.03 ± 0.13 (n = 11)	0.27 ± 0.04 (n = 11)	0.91 ± 0.15 (n = 11)
<i>p</i> Value	0.31	0.87	0.97	0.21	0.37	0.72	0.11

Values are means ± SD. The *p* values for group comparisons are derived from *t*-test.

* *p* < 0.05.

MRS = MR spectroscopy; NAA = *N*-acetyl aspartate; Cr = total creatine; Cho = choline-containing compounds; Ins = myo-inositol.

metabolites and ratios. There were no significant differences in any brain region. For the patients with a history of neuropsychological or neurodevelopmental difficulties, there was a decreased Cho/Cr in the LFW (0.32 ± 0.08 vs 0.42 ± 0.06 ; *p* = 0.01) and an increased Ins/Cr in the RBG compared with controls (0.58 ± 0.11 vs 0.47 ± 0.07 ; *p* = 0.03).

Neuropsychological assessments. As shown in table E-2 in the supplementary material on the *Neurology* Web site, HIV patients and control subjects had similar performance on the panel of tests except for spatial learning and memory using the CMS Total score. The *p* value was marginally significant using *t*-test (*p* = 0.045), with a score of 8.8 ± 2.6 for patients and 11.0 ± 3.0 for control subjects.

MRS metabolites vs age. Significant correlation coefficients between metabolite concentrations and age are shown separately for patients and for control subjects in

table 2. Additional correlation coefficients that showed trends for significance are also listed in table 2. [NAA] in three brain regions (RFW, LFW, RHIP) correlated with age in control children but not in HIV-infected patients (see table 2; also see figure E-1 in the supplementary material on the *Neurology* Web site). The differences in the age-related increase in [NAA] between the control subjects and the HIV patients are significant in both the LFW and the RHIP. These results are consistent with a significant developmental impact of HIV infection on neuronal growth. The RHIP [NAA]/[Cr] similarly increased with age only in the control subjects (*r* = 0.73, *p* = 0.01). Figure E-1 on the *Neurology* Web site demonstrates linear regression for these parameters in these brain regions.

In contrast, HIV subjects but not control subjects showed strong correlations between age and [Ins] in both

Table 2 Correlations between cerebral metabolite concentrations and age in patients and controls

Parameters	Patients <i>r</i> (<i>p</i>)	Controls <i>r</i> (<i>p</i>)	Fisher <i>z</i> - transformation <i>p</i> value
[NAA]			
RFW vs age	0.08 (0.74)	0.59 (0.04)*	0.15
LFW vs age	-0.25 (0.33)	0.66 (0.02)*	0.01*
RHIP vs age	-0.30 (0.32)	0.69 (0.02)*	0.02*
[NAA]/[Cr]			
RHIP vs age	-0.50 (0.08)	0.73 (0.01)*	0.002*
[Ins]			
RFW vs age	0.71 (0.0006)*	0.32 (0.41)	0.24
LFW vs age	0.65 (0.006)*	0.10 (0.77)	0.13
RFW vs age (Rx)	0.62 (0.005)*		
[Ins]/[Cr]			
RFW vs age	0.66 (0.002)*	0.16 (0.68)	0.19

* $p < 0.05$ for either Pearson correlation coefficient or Fisher z transformation comparing correlations for patients and control subjects.

NAA = *N*-acetyl aspartate; RFW = right frontal white matter; LFW = left frontal white matter; RHIP = right hippocampus; Cr = total creatine; Ins = myo-inositol; Rx = treatment.

the RFW and the LFW ($r = 0.71$, $p = 0.0006$ and $r = 0.65$, $p = 0.006$; see table 2 and figure E-2 on the *Neurology* Web site). RFW [Ins]/[Cr] also correlated with age ($r = 0.66$, $p = 0.002$) in HIV patients only, but the difference in correlation coefficients between patients and control subjects was not statistically significant. In addition, RFW [Ins] in HIV-infected children correlated with age at treatment initiation for HIV ($r = 0.62$, $p = 0.005$; see table 2 and figure E-2 on the *Neurology* Web site); this finding indicates that children treated for HIV later in life had higher [Ins] in the RFW.

Correlation coefficients for metabolites vs \log_{10} plasma viral load were also determined. The \log_{10} viral load correlated positively with RFW [Ins] ($r = 0.54$, $p = 0.02$) and with MFG [Cho] ($r = 0.49$, $p = 0.04$) but negatively with RBG

[Ins] ($r = -0.52$, $p = 0.05$) and with RFW [Cho]/[Cr] ($r = -0.50$, $p = 0.03$) (see figure E-3 on the *Neurology* Web site).

Metabolites vs neuropsychological assessments. Table 3 summarizes the metabolite and neuropsychological variables that showed significantly different correlation coefficients in patients vs control subjects. Specifically, spatial memory testing after long delay (CMS Long Delay) correlated positively with RHIP [Cho] in the patients ($r = 0.68$, $p = 0.02$) but not in control subjects. The difference in correlation coefficients was significant ($p = 0.03$; see table 3 and figure E-4 on the *Neurology* Web site).

For tests of motor function, control subjects demonstrated a strong positive correlation between RFW [Ins]/[Cr] and the Purdue Pegboard Dominant Test ($r = 0.86$, $p = 0.006$) as well as between the RHIP [Ins] and the Purdue Pegboard Nondominant Test ($r = 0.68$, $p = 0.03$). In contrast, patients showed no significant correlation (RFW [Ins]/[Cr] vs Purdue Dominant) or a negative correlation (RHIP [Ins] vs Purdue Nondominant; $r = -0.58$, $p = 0.048$). Hence, correlation coefficients between the two groups were significantly different for [Ins]/[Cr] vs Purdue Dominant ($p = 0.002$) and for RHIP [Ins] vs Purdue Nondominant ($p = 0.003$) (see figure E-4 on the *Neurology* Web site).

CDI Total, a self-report of depressive symptoms, correlated negatively with [Cho]/[Cr] in the RFW of the control subjects ($r = -0.78$, $p = 0.01$) (see figure E-5 on the *Neurology* Web site). This correlation was not present in the patients, and the group difference was significant (Fisher z transformation, $p = 0.03$). Similar results were obtained for [Cho] in RFW vs CDI Total ($r = -0.75$, $p = 0.02$) for control subjects; however, this correlation was not significantly different between the two groups. The control subjects who had higher CDI scores were more depressed and had lower [Cho]/[Cr] and [Cho] in the RFW.

EOWPVT-R correlated positively with LFW [Ins] in the control subjects ($r = 0.67$, $p = 0.03$) but negatively with RFW [Ins]/[Cr] ($r = -0.54$, $p = 0.02$) in the patients; both of these correlations were different between groups ($p < 0.05$) (see table 3 and figure E-5 on the *Neurology* Web site). In contrast, both RFW [Ins] and RFW [Ins]/[Cr] correlated inversely with performance on the CMS Dot Location Long Delay (see figure E-6 on the *Neurology* Web site) for both patients as well as control subjects.

Table 3 Cerebral metabolites vs neuropsychological assessments

Parameters	Patients <i>r</i> (<i>p</i>)	Controls <i>r</i> (<i>p</i>)	Fisher <i>z</i> -transformation <i>p</i> value
CMS Long Delay vs RHIP [Cho]	0.68 (0.02)*	-0.25 (0.49)	0.03*
Purdue Pegboard Dominant vs RFW [Ins]/[Cr]	-0.33 (0.18)	0.86 (0.006)*	0.002*
Purdue Pegboard Nondominant vs RHIP [Ins]	-0.58 (0.048)*	0.68 (0.03)*	0.003*
EOWPVT vs RFW [Ins]/[Cr]	-0.54 (0.02)*	0.474 (0.24)	0.03*
vs LFW [Ins]	-0.37 (0.17)	0.67 (0.03)*	0.01*
CDI vs RFW [Cho]/[Cr]	0.04 (0.88)	-0.78 (0.01)*	0.03*

* Significant ($p < 0.05$) for either Pearson correlation coefficient (r) or Fisher z -transformation comparison of correlation coefficients (r) for patients and control subjects.

CMS = Children's Memory Scale; Cho = choline; RHIP = right hippocampus; INS = myo-inositol; Cr = creatine; RFW = right frontal white; EOWPVT = Expressive One Word Picture Vocabulary Test; LFW = left frontal white; CDI = Children's Depression Inventory.

Discussion. Our most striking finding is that HIV-infected children do not demonstrate a normal age-associated increase in [NAA], a neuronal marker, in the frontal white matter and hippocampus. These results are consistent with a significant developmental impact of HIV infection on neuronal growth and demonstrate the severity of the developmental insult from early infection with HIV as 19 of our 20 patients were infected as neonates from their mothers. As an increase in absolute [NAA] with age for frontal white matter and hippocampus has not been reported previously for normal children ages 6 to 16, these findings were surprising, although several investigators have reported age-associated changes in metabolite ratios during childhood and adolescence.²⁶⁻²⁹ Previous investigators²⁶ noted that [NAA]/[Cho] increased linearly with age in white matter between 3 and 19 years of age. Similarly, there are reported age-associated increases in [NAA]/[Cho] in the centrum semiovale white matter up to age 18.5 years,²⁷ whereas another report²⁹ noted increases of [NAA]/[Cho] and [NAA]/[Cr] in the paraventricular region during childhood. Our findings of increasing [NAA] during childhood and adolescence in the frontal white matter of the control children would be consistent with these earlier observations.

Decreased [NAA] has been reported in adult patients with moderate to severe HIV dementia but not in those who were asymptomatic or had mild dementia,^{4,6} and decreased [NAA]/[Cr] in both white matter¹⁸ and basal ganglia¹⁹⁻²¹ of children with HIV encephalopathy also has been reported. We did not find decreased [NAA] in our patients as a group compared with the control subjects; this might reflect the general well-being of our patient population and the fact that most children had relatively low viral load (<5,000 HIV RNA copies/mL).

For HIV-infected patients, RFW [Ins] correlated positively with both age at treatment initiation and age at time of MRS testing. The older children were diagnosed and treated at a later age and did not have the advantage of current early diagnosis and aggressive treatment strategies in infancy. The age-related increases in the glial marker [Ins] suggest increased glial proliferation with age or more severe inflammatory response to HIV infection in the older children. Increased [Ins] is a typical finding in adults with HIV brain involvement,^{4,7,11,12,14} and one pediatric study¹⁸ has also reported increased [Ins] in white matter of children with HIV encephalopathy. As cerebral atrophy with ventriculomegaly and extensive gliosis of the white matter has been documented pathologically in children with HIV,³⁰ our findings of increased [Ins] in the frontal white matter with age is consistent with prior studies. Only careful metabolite testing of children diagnosed and treated with HAART in infancy will determine if the striking age-related effect on [NAA] and [Ins] that we found can be avoided with earlier diagnosis and treatment.

Reassuringly, metabolite concentrations and metabolite ratios were similar for patients and control subjects with the exception of [Cho], a marker of cell

membrane integrity, in the LFW. In particular, the subgroup of patients with a higher viral load (>5,000 HIV RNA copies/mL) had 15% lower [Cho] in the basal ganglia than the control subjects. It has been previously reported²¹ that [Cho]/[Cr] was decreased in the basal ganglia only for HIV-infected children without evidence of HIV encephalopathy. Therefore, our results suggest that the decreased Cho/Cr observed by these previous investigators is due primarily to decreased [Cho]. As the basal ganglia and frontal white matter are both areas of the brain known to be significantly affected by HIV infection in children,^{30,31} lower [Cho] may indicate cell loss in these regions.³²

Our finding of decreased [Cr] (-13%; $p = 0.02$) in the basal ganglia of patients with higher viral load compared with control subjects is consistent with prior observations of decreased [Cr] in the basal ganglia of adult HIV patients with severe dementia.¹⁵ As [Cr] is present in both neurons and glia, the decreased level in the basal ganglia of these patients is also consistent with cell loss, possibly due to a greater degree of neurotoxic viral protein-mediated apoptosis. Our findings of multiple decreased metabolite concentrations ([Cho], [Cr], and [Ins]) in the RBG for children with high viral load compared with control subjects or children with lower viral load demonstrate the significant involvement of the basal ganglia in HIV-infected children.

The higher [CHO] in the MFG (+25%; $p = 0.002$) of patients with higher viral load compared with those with lower viral load is consistent with findings in adult patients with early stages of brain injury.⁸ Some investigators also found increased [Cho] in asymptomatic adult HIV patients, whereas others have documented increased [Cho] or [Cho]/[Cr] in HIV patients with neurologic findings.^{5-9,12,13,16} There are no previous reports of increased [Cho] in MFG for pediatric HIV patients. As glial cells have much higher [Cho] than neurons,³ the increased [Cho] suggests glial activation in the MFG of these HIV-infected children.

Neuropsychological assessments were not significantly different between patients and control subjects except that HIV-infected children performed more poorly on the CMS test of spatial memory. The lower performance on spatial memory may be significant because [Cho] in the hippocampus correlated positively with the delayed spatial memory testing in the patients ($r = 0.68$) but not in the control subjects. As the hippocampus has known important functions in memory and particularly spatial memory,³³⁻³⁵ these results suggest a biologic relevance. Those with less cell loss in the hippocampus and resultant higher [Cho] appeared to have better spatial memory. Recent work has suggested the possible selective vulnerability of hippocampal neurons to HIV-related injury,³⁶ although others have not found hippocampal neuronal loss in AIDS patients.³⁷ Furthermore, some reported increased HIV RNA viral load in the basal ganglia and hippocampus compared with the

cerebellar cortex and midfrontal cortical gray matter.³⁸ Our observation regarding spatial memory performance correlating with [Cho] in the RHIP of HIV-infected children needs further confirmation with enhanced techniques, such as two-dimensional MRS,³⁹ to further characterize the functional significance of the different components of the Cho peak.

Significant differences between the control children and those infected with HIV were observed for several other correlations between brain metabolites and cognitive performance. These findings suggest that the relationships between cerebral metabolite and neuropsychological test results might be altered by HIV infection. Therefore, brain metabolite measurement might provide insights into how HIV infection might affect brain function.

One potential confound in our study is that some of the children were exposed in utero to maternal substance abuse. Past maternal drug abuse history was available for only 12 of our 20 HIV patients. As only 3 of these 12 patients had mothers with a history of drug abuse, it was not possible to determine the potential effects of maternal substance abuse on brain metabolite levels. Prenatal exposure to methamphetamine or cocaine in utero would have led to increased [Cr] in the basal ganglia⁴⁰ or the frontal white matter,⁴¹ but our patients had decreased [Cr]. Another area of inadequate data is the reporting of the highest previous plasma viral load. Although we recorded the highest known viral load, many of these children were diagnosed with HIV infection prior to the use of viral load testing. Therefore, we were not able to correlate cerebral metabolite concentration with known previous highest viral load. Future studies should correlate previous highest viral load as an indicator of past severity of infection and determine whether it would have any impact on cerebral metabolite values subsequently.

Our striking findings of age-associated effects on [NAA] and [Ins] are very concerning regarding the developmental impact of HIV on [NAA] and the effects of long-term HIV infection on [Ins]. As encephalopathy is a significant problem for many surviving HIV-infected children (16% after 7 years of survival),⁴² there is a continued need to improve early detection of CNS damage when intervention such as change of medication may still be possible. The age-related effects of HIV infection in our cohort may not be observed in future HIV-infected children who receive early and aggressive antiretroviral regimens in infancy.

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