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Neuropsychological Function and Cerebral Metabolites in HIV-infected Youth

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Abstract

The effects of HIV on brain metabolites and cognitive function are not well understood. Sixteen HIV+ youths (15 vertical, 1 transfusion transmissions) receiving combination antiretroviral therapy and 14 age-matched HIV- youths (13–25 years of age) were evaluated with brain two-dimensional (2D) magnetic resonance spectroscopy (MRS) at 3 Tesla (T) and a neuropsychological battery that assessed three cognitive domains (attention/processing speed, psychomotor ability, and executive function). The relationship between brain metabolite ratios and cognitive performance was explored. Compared to HIV- controls, HIV+ subjects had higher scyllo-inositol (Scy)/total creatine (tCr) (+32%, $p=0.016$) and higher Scy/total choline (tCho) (+31%, $p=0.018$) on 2D-MRS in the right frontal lobe. HIV+ subjects also had higher glutamate (Glu)/tCr (+13%, $p=0.022$) and higher Glu/tCho (+15%, $p=0.048$) than controls. HIV+ subjects demonstrated poorer attention/processing speed ($p=0.011$, $d=1.03$) but similar psychomotor and executive function compared to HIV- controls. The attention/processing score also correlated negatively with the ratio of N-acetylaspartate (NAA) to tCr on 2D-MRS ($r=-0.75$, $p=0.0019$) in the HIV- controls, but not in the HIV+ subjects (Fisher's r -z transformation, $p<0.05$). Our results suggest that attention/processing speed is impacted by early HIV infection and is associated with right hemisphere NAA/tCr. Scy and Glu ratios are also potential markers of brain health in chronic, lifelong HIV infection in perinatally infected youths receiving antiretroviral therapy.

Keywords

HIV; MR Spectroscopy; Brain metabolites; Glutamate; Scyllo-inositol; Cognitive domains

Introduction

Many perinatally HIV-infected youths have now survived to adolescence and adulthood due to effective antiretroviral therapies. Since these patients acquired HIV at a time of relative immune compromise (*in utero* and at birth), signs of neurocognitive compromise are common (Tardieu et al. 1995) despite the dramatic decrease in the diagnosis of HIV encephalopathy in the era of effective antiretroviral therapy (Patel et al. 2009). Assessment of the neurocognitive function of these youths as they continue to survive for many years has become an important area of research, since sensitive early detection of neurocognitive or neurologic compromise might result in treatment modifications that could ultimately improve their brain health and function. Magnetic resonance spectroscopy (MRS) can noninvasively assess cerebral metabolites and brain health in HIV-infected patients.

One dimensional (1D) proton (^1H) MRS is typically recorded using 1.5 Tesla (T) scanners to study cerebral metabolites. Although standardized and easily performed, the technique can reliably detect only few neurochemicals due to spectral resolution and time constraints: N-acetylaspartate (NAA, a neuronal marker), soluble choline-containing compounds (Cho, a marker of membrane turnover, myelination or myelin break down), total creatine (Cr, a marker of energy metabolism), and myo-inositol (mI- a glial cell marker). Studies in adult

HIV-infected subjects have shown metabolite abnormalities compared to seronegative controls in both the grey and white matter. In particular, HIV patients with cognitive and motor impairment may have decreased NAA or NAA/Cr, increased Cho or Cho/Cr, and increased mI or mI/Cr (Laubenberger et al. 1996; Lopez-Villegas et al. 1997; Chong et al. 1993; Möller et al. 1999). Abnormalities in metabolite concentrations were correlated with impaired fine motor and psychomotor function, as well as deficits in executive function in HIV patients (Chang et al. 2002).

The few MRS studies in perinatally HIV-infected children also found similarly decreased NAA and elevated mI in the white matter (Salvan et al. 1998), as well as decreased NAA/Cr in the basal ganglia of the children with HIV-encephalopathy (Pavlakis et al. 1995, 1998). In addition, decreased Cho/Cr in the basal ganglia of HIV-infected children without a diagnosis of encephalopathy was also reported (Lu et al. 1996). Similarly, using 1D-MRS, we found lower Cho concentrations in the left frontal white matter (in contrast to adult findings) and no differences in absolute concentration of NAA or mI in five different brain regions from a group of clinically-stable HIV-infected children compared to seronegative controls (Keller et al. 2004). Furthermore, the HIV-infected children did not show the normal age-associated increase in the neuronal marker NAA, both in the frontal white matter and hippocampus, while the glial marker mI increased with age suggesting delayed brain development and greater neuroinflammation only in the HIV-infected children. Those with higher mI in the frontal white matter also were treated with antiretroviral medications at an older age, suggesting that children with delayed treatment might have more ongoing neuroinflammation. Since these children were relatively stable clinically, no significant changes in these limited neurometabolites were found over a 10-month longitudinal period (Keller et al. 2006).

Two-dimensional (2D) MRS converts a crowded, overlapping 1D-MRS spectrum to a better resolved 2D spectrum through the addition of a 2nd spectral dimension (Thomas et al. 2001) which allows the measurements of many more neurochemicals. The time interval between the second 180° and third 90° radiofrequency (RF) pulses is incrementally increased to encode the frequencies along the second dimension. In our earlier 2D MRS study of HIV-infected youths, we used a 1.5 T scanner with a four-channel bitemporal phased-array coil for reception and successfully detected 11 metabolites in the left frontal brain region (Banakar et al. 2008). Metabolite ratios, mI/Cr and mI/Cho, were both elevated in the frontal brain of HIV-infected children and youths 9–21 years of age, which suggested ongoing neuroinflammation similar to those found in earlier studies of HIV-infected youths and children. To further investigate neurometabolite abnormalities in HIV-infected youths, we implemented the 2D localized correlated spectroscopy (L-COSY) technique, on a 3T MRI/MRS scanner and used a 12 channel phased array head receiver coil. This technique permits detection of even more neurometabolites on the 3T MRI scanner. To evaluate the consequences of neurometabolites abnormalities, we also explored the relationship between the metabolite ratios and cognitive performance in the HIV-infected youths and their uninfected controls. Several investigators reported neurocognitive deficits in children with perinatal HIV infection (Tardieu et al. 1995; Keller et al. 2004; Koekkoek et al. 2008; Martin et al. 2006; Blanchette et al. 2002; Bisiacchi et al. 2000; Wolters et al. 1995), but only two studies evaluated the relationship between neurometabolites levels and cognitive performance. A study in a small number of HIV-infected children (mean age 11.8 years) found that higher NAA/Cho ratio was associated with better arithmetic and comprehension abilities (Gabis et al. 2006). In our earlier 1D-MRS study (Keller et al. 2004), we also found that the choline concentration in the hippocampus of HIV+ children (6–16 years of age) correlated with performance on a delayed spatial memory test. The present study extends earlier observations by studying a group of antiretroviral treated HIV+ youths with lifelong

and longer duration of infection, using a targeted neuropsychological test battery and the 2D L-COSY technique at a higher magnetic field (3T).

Material and methods

Research design

This study received approval from both Institutional Review Boards at Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center and at the University of California at Los Angeles. All subjects completed study procedures voluntarily. Signed informed consent was obtained from all subjects 18 years or older and from the parents or legal guardians of younger subjects. Subjects between the ages of 13–17 years provided signed assent to participate.

Subjects—Sixteen HIV-infected youths were recruited from four medical centers providing care for perinatally HIV-infected youths in the Los Angeles area: Los Angeles County Harbor-UCLA Medical Center (Torrance, CA), David Geffen School of Medicine at UCLA (Los Angeles, CA), Miller's Children's Hospital of Long Beach (Long Beach, CA) and Children's Hospital Los Angeles (Los Angeles, CA). In addition, 14 control subjects were recruited from family members or friends of the subjects and from the general pediatric clinic at Los Angeles County Harbor-UCLA Medical Center. Inclusion criteria were: 1) 13–25 years of age; 2) acquisition of HIV in fetal or neonatal period or in first year of life by blood transfusion (for HIV+ subjects) or, confirmation of HIV-uninfected status with Ora-Quick (OraSure Technologies, Bethlehem, PA 18015) buccal scraping (for HIV– subjects); 3) if HIV-infected, confirmation of current treatment with combination antiretroviral medication; 4) post menarchal status for all females since all females were studied in the follicular phase of the menstrual cycle to avoid the menstrual changes associated with cerebral metabolites; 5) right-hand dominance. Exclusion criteria were: 1) opportunistic central nervous system infection or any central nervous system diseases (other than HIV in the HIV+ group); 2) metabolic disturbances; 3) metallic implants or braces; 4) claustrophobia; 5) Attention Deficit/Hyperactivity Disorder; 6) pregnancy (by urine test before scanning); 7) nicotine, alcohol or other substance use/abuse; 8) active depression, anxiety, signs of other current known psychiatric diagnosis; 9) severe school difficulties in control subjects; 10) chronic medication other than asthma medication in control subjects.

Each subject fulfilling the study criteria was assessed with a battery of neuropsychological tests and 2D MRS. For HIV+ subjects, the following additional data were collected from chart review: age at first treatment for HIV, HIV viral load at time of testing, CD4 T cell counts at time of testing, current antiretroviral therapy, and history of maternal substance abuse during pregnancy.

Two-dimensional MRS method

Two-dimensional MRS examination was performed on a Siemens 3T MRI/MRS scanner (Magnetom Trio-Tim, Siemens Medical Solution, Erlangen, Germany) using the standard 12 channel head phased array 'receive' coil. Axial T₁-weighted MR images were used to select a volume of interest (VOI) from which the 2D MRS was acquired. A WET (water suppression enhanced through T₁ effects) (Ogg et al. 1994) method with three frequency selective RF pulses was used for global water suppression. The fast automatic shimming technique by mapping along projections (FASTMAP) (Gruetter 1993) was successfully used in order to improve the B₀-homogeneity over the localized voxel. The full-width-at-half maximum (FWHM) of water resonance was typically 15 Hz in the right frontal lobe containing both gray and white matter.

The 2D L-COSY sequence consisted of three slice-selective RF pulses for the VOI localization, similar to 1D PRESS (Bottomley 1987), but the last 180° pulse was replaced by 90°, with the last 90° RF pulse also enabling the coherence transfer necessary (Thomas et al. 2001). The spectral encoding for the second dimension was inserted between the second and third slice selective RF pulses. 2D L-COSY spectra were recorded using the following parameters: Initial echo time (TE) = 30 ms, Repetition time (TR) = 2000 ms, 800 scans (100 Δt_1 increments to encode the 2nd spectral dimension and 8 excitations per Δt_1 for signal averaging), with voxel size of 27 cm³ and a total scan time of ~26 min. The 2D raw matrix consisted of 2048 complex points along the first and 100 points along the second dimensions. The data were processed using MATLAB based prior knowledge fitting (ProFit) algorithm (Lange et al. 2008; Frias-Martinez et al. 2008; Nagarajan et al. 2012). 2D L-COSY spectra were recorded from all 14 control subjects and 15 of the 16 HIV-infected subjects (data from one subject was excluded due to poor quality which was due to subject's movement). The 2D L-COSY spectra were recorded from the right frontal white/gray matter region only.

Prior knowledge generated for L-COSY included 20 metabolites: total creatine and phosphocreatine (tCr), N-acetylaspartate (NAA), glycerylphosphocholine (GPC), phosphocholine (PCh), free choline (Cho), alanine (Ala), aspartate (Asp), γ -aminobutyrate (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), glutathione (GSH), lactate (Lac), myo-inositol (mI), N-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), taurine (Tau), scyllo-inositol (Scy) and ascorbate (Asc). The 2D L-COSY spectra were then processed using the modified ProFit code and the measurement accuracy was characterized using CRLB (Cavassila et al. 2001; Nagarajan et al. 2012). Criteria for rejection of spectra or individual metabolite values were based on CRLB > 20%, patient movement, or poor water suppression. The 2D MRS results are reported as ratios of metabolites with respect to tCr and tCho.

Neuropsychological testing

Each subject was evaluated with a neuropsychological test battery that assessed three cognitive domains: attention/processing speed, psychomotor function, and executive/problem solving skills. This battery comprised the following tests: Conners' Continuous Performance Test II version 5 (CPT II; Conners and MHS Staff 2000) (CPT-II; a measure of sustained attention), Purdue Pegboard Test (Yeudall et al. 1986) (a test of psychomotor function) and the Color-Word-Interference Test and Trail Making Test subtests of the Delis-Kaplan Executive Function System (Delis et al. 2001) (DKEFS; measures of attention and executive ability). Domain scores of attention/processing speed, psychomotor function, and executive ability were theoretically determined and informed by the psychometric properties of the tests in our battery. The attention/processing speed *T*-score was derived by averaging *T*-scores of the DKEFS Color-Word-Interference Test CWC1 (Color Test) and CWC2 (Word Test) *T*-scores and the reversed *T*-scores for the CPT-II's Omission, and Hit Reaction Time indices. The psychomotor score was obtained by calculating the average of the *T*-scores for the Purdue Pegboard's Right Hand and Assembly *T*-scores, and *T*-scores for three of the DKEFS Trail Making Test subtests (C2, C3, and C5). The executive/problem solving score represents the average of the *T*-scores for DKEFS' Trail Making Test subtest C4, the Tower Test achievement score and the movement accuracy ratio, and the inhibition and switching trials of the Color-Word-Interference Test (CWC3 and CWC4, respectively).

Although neurocognitive testing was performed within 2 months of the MR scans for 15 of 16 HIV+ subjects, one HIV+ subject was studied at 74 days due to scheduling issues. Similarly for the HIV- subjects, neurocognitive testing followed the scans within 2 months for 12 of 14 subjects and 119 days and 133 days for two HIV- subjects.

Statistical methods

Group neuropsychological test performances were compared with *t*-tests using SAS, version 9.2 (SAS Institute, Inc, Cary, NC). An analysis of covariance was performed for 2D MRS ratios with age as a covariate to control for possible age associated effects on the metabolites. Cohen's *d* effect size estimates were also calculated (Becker 2011). Metabolite ratios for 2D-MRS were correlated with the 3 domain *T*-scores using Pearson's correlation coefficient. Correction for multiple comparison for correlations was made using SAS step-down Bonferroni test to adjust *p* values. Correlations were compared between HIV+ and HIV-groups using Fisher's *r*-to-*z* transformation.

Results

Subject characteristics

Demographic information for the subjects is summarized in Table 1. All HIV+ subjects were receiving combination antiretroviral therapy with at least three medications and were clinically stable. One HIV+ subject was infected by a blood transfusion in the first year of life while the remainder HIV+ subjects were infected from their mothers. Of the HIV-infected subjects, one had mild IgA nephropathy, one had stable untreated chronic hepatitis C infection, and one additional subject had a previous diagnosis of bipolar disorder, but did not currently display signs of mood fluctuations and was not receiving treatment for this condition per parental report. The HIV+ subjects initiated their antiretroviral treatment at a wide age range, from 4 months to 14.5 years. Of the 16 HIV-infected subjects, 13 were receiving antiretroviral therapy that included protease inhibitors. The three subjects who were not receiving protease inhibitors were receiving nonnucleoside reverse transcriptase inhibitor and/or integrase inhibitor based regimens. Nine of the 16 HIV-infected subjects had evidence of neurocognitive problems prior to study participation based on previous clinical diagnosis of HIV encephalopathy, prior documented poor performance on neuropsychological tests, or clinical history of developmental delay. Poor school performance as evidenced by delayed graduation from high school or an individual education plan in school suggested possible HIV-associated neurocognitive problems for two other subjects. Thus, the majority (11/16) of these HIV+ youths had some previous evidence of nervous system involvement likely related to HIV-infection.

Neurocognitive assessments

Table 2 shows the composite *T*-scores for the three cognitive domains for each group and the group differences. HIV+ subjects performed poorer than the HIV- subjects on the attention/processing speed domain *T*-score ($p=0.011$, $t=2.715$, Cohen's $d=1.03$). The psychomotor score and the executive/problem solving score were not significantly different between the two groups. Figure 1 shows the distribution of the attention/processing speed, psychomotor, and executive problem solving *T*-scores for HIV+ and HIV- subjects. Box indicates 25th and 75th percentile and extenders indicate range. These plots show lower distribution of the scores in the HIV+ subjects compared to HIV- controls.

2D- MRS in the right frontal lobe voxel

Figure 2a and b show the 2D L-COSY spectra of an 18 year old healthy control and a 17 year old HIV+ patient. The 2D L-COSY diagonal peaks come from dominant singlets due to NAA_d (2.0, 2.0 ppm), Cr_d (3.0, 3.0 ppm), and Cho_d (3.2, 3.2 ppm) and these were single resonances due to the respective methyl groups of Cr and NAA, and the trimethyl groups of Cho which correspond to the peaks usually observed in 1D-MRS. The remaining peaks in the spectra are 2D cross peaks of metabolites which show resonances from overlapping severely in 1D-MRS. These were clearly separated in the 2D L-COSY spectra. The

advantage of using 2D spectroscopy is to identify many metabolite peaks (NAA, mIScY, mICh, Glu/Gln, Asp, PE, GSH, Thr/Lac, GABA, GPC/PCh and Tau) that were identified below the diagonal peaks less ambiguously than 1D MRS. The locations of the cross peaks in a 2DL-COSY spectrum in the F2 and F1 dimensions were similar to what was reported in the previous studies (Thomas et al. 2001; Thomas et al. 2003; Banakar et al. 2008; Nagarajan et al. 2012): NAA (4.3,2.6 ppm), Glu/Gln (3.7, 2.0 ppm), mICh (4.0,3.5, ppm), mI (3.5,3.1 ppm), Scy (3.3, 3.3 ppm), Asp (2.7,3.9 ppm), PE (4.0,3.2 ppm), GSH (4.5, 2.9 ppm), Lac/Thr (4.1,1.3 ppm), GABA/MM (2.9,1.9 ppm), GPC/PCh (4.3, 3.6 ppm) and Tau (3.4, 3.2 ppm). As reported previously, overlap of peaks (mI and Cho, mI and Scy, etc.) is a problem even in 2D MRS. Using the prior-knowledge fitting, 2DMRS quantitation of these overlapping and other metabolites was accomplished here (Thomaset al. 2003;Banakar et al. 2008;Nagarajan et al. 2012). Table 3 shows the metabolite ratios with respect to tCho and to tCr. Only 2 sets of metabolite ratios, Scy and Glu, showed group differences between HIV+ subjects and controls relative to tCr or tCho. Compared to HIV- controls, HIV+ subjects had higher Scy/tCr (+32%, $p=0.016$) and higher Scy/tCho (+31%, $p=0.018$), showing consistency between the two ratios. Similarly, compared to the HIV- controls, HIV+ subjects had moderately elevated Glu/tCr (+13%, $p=0.022$) and Glu/tCho (+15%, $p=0.048$). Scy or Glu ratios did not correlate with the % CSF within the right frontal voxel ($2.17\% \pm 1.28\%$ for control subjects and $2.44\% \pm 1.48\%$ for HIV+ subjects). In addition, several additional metabolites ratios were higher in the HIV subjects than controls: Gln+ Glu/tCr (+11.5%, $p=0.049$); Asp/tCr (+18.6%, $p=0.048$). mI/tCr also showed a trend to be higher (+22%) in HIV+ subjects than controls ($p=0.057$).

Relationship between metabolite ratios and cognitive domain T-scores

Since several metabolite ratios on 2D-MRS showed group differences in the right frontal lobe, we evaluated how metabolite ratios might contribute to cognitive performance on the three domain T-scores. A strong negative correlation was seen between NAA/tCr and attention/ processing speed T-score in the control subjects only ($r=-0.75$, $p=0.002$), but not for the HIV+ subjects. This difference in the correlations between the controls and HIV+ subjects was significant (Fisher's r -z transformation $p<0.05$). However, neither NAA/tCr nor any of the other metabolite ratios correlated with the domain T-scores for psychomotor function and executive functioning in the controls or in the HIV+ subjects.

Correlations between age of first antiretroviral treatment, age at time of scan, CD4 count and viral load with cognitive domain scores and metabolite ratios

In the HIV subjects, earlier age of first ARV treatment tended to be associated with better cognitive domain T-scores, but none of these correlations reached statistical significance after correction for multiple comparisons (age of first treatment and attention processing: $r=-0.14$.; psychomotor: $r=-0.54$, executive function: $r=-0.18$). Similarly, their cognitive performance in these three domains did not correlate with their CD4 count (attention/ processing: $r=-0.22$, psychomotor: $r=-0.17$, executive: $r=-0.34$) or with their log viral load (attention/processing: $r=-0.03$, psychomotor: $r=0.19$, executive: $r=-0.14$). None of the metabolite ratios correlated with age of first treatment for HIV, age of scan, CD4%, CD4 count or log viral load, after correction for multiple comparisons.

Discussion

This study evaluated the neurocognitive function and 2D-MRS cerebral metabolite ratios of older, treated, HIV-infected youths who were infected perinatally or in the first year of life as compared to HIV- control subjects. Attention/processing speed was poorer in the HIV-infected youths than the HIV- controls. Although we were able to demonstrate higher than normal levels of Scy and Glu with respect to both tCr and tCho in the right frontal brain of

the HIV+ subjects in comparison to the HIV– controls, we could not demonstrate a correlation of these metabolites with neurocognitive performance.

Previous studies of neurocognitive function in perinatally HIV-infected children have reported on performances in younger children than in our study (Tardieu et al. 1995; Koekkoek et al. 2008; Blanchette et al. 2002; Bisiacchi et al. 2000; Wolters et al. 1995) and have revealed deficits in language, visual–spatial and visuo–praxic skills, psychomotor ability, executive function, and memory; although many cognitive performances were within the normal range and intellectual facility at times was average. In 2008, Koekkoek et al. found that HIV– infected children performed more poorly in executive functioning (attentional flexibility and visuospatial working memory) and processing speed compared to age appropriate norms. A recent study (Smith et al. 2012) of perinatally HIV-infected children 7–16 years of age showed that children with an AIDS diagnosis performed poorer than children without an AIDS diagnosis in full-scale intelligence quotient, perceptual reasoning and working memory/processing speed, attributable to a previous diagnosis of encephalopathy. Thus, these previous studies are generally consistent with our results in that attention and processing speed appears to be a sensitive indicator for this perinatally infected group and that these cognitive abnormalities persist as these children reach adolescence and adulthood.

Studies of neurocognitive impairment in adults with HIV suggest deficits in attention/speeded processing, psychomotor abilities, memory, and executive functioning (Hinkin et al. 1998; Miller et al. 1990; Selnes et al. 1995; Heaton et al. 1995). However, recent studies in adults (Heaton et al. 2011) have shown a shift in neurocognitive profile in the post-combination antiretroviral era with more deficits in executive function and learning and less deficits in motor, verbal, and speed of information processing functions.

Our most consistent metabolite observations were the elevation of Scy and Glu ratios with respect to both tCr and tCho. As reviewed by Seaquist et al. (Seaquist and Gruetter 1998) although the function of it is not known, Scy can rearrange to form mI, the precursor to myo-inositol-1-phosphate which is important in signal transduction and membrane integrity. Meyerhoff et al. (Meyerhoff et al. 1996) reported that they had observed a Scy peak in two HIV– infected adults, one with severe alcoholism and the other with significant psychiatric disease and mild alcoholic intake. The relationship to HIV is unclear since Scy was subsequently reported by Viola et al (Viola et al. 2004) as a new marker of brain metabolism disturbances induced by chronic alcoholism. Alcoholism was an exclusion criterion for our study so it was not a confounding variable for our analysis.

Scy/Cr has been found (Griffith et al. 2007) to be elevated in the brains of Alzheimer's patients compared to healthy controls and Scy/Cr ratios correlated with a poorer performance on a clock drawing measure which suggested that elevated Scy/Cr was associated with more impairment. Kaiser et al. (Kaiser et al. 2005) have found higher levels of Scy and mI in the corona radiata of older individuals. Taken together these findings from prior studies suggest that the 31–32% higher than normal cerebral Scy in our perinatally infected HIV+ subjects likely reflects negative brain health.

Our other metabolite observation was the 13–15% higher than normal glutamate to tCr and tCho ratios in the frontal lobe of HIV youths. Glutamate mediates most of the fast excitatory neurotransmission in the central nervous system and is the principal mediator of sensory information, motor coordination, emotions and cognition, and both memory formation and retrieval (Hassel and Dingledine 2006). Glutamate excitotoxicity has been explored as a mechanism in the neuropathogenesis of HIV since excess glutamate may result from impairment of astrocytic reuptake of glutamate by HIV envelope glycoprotein 120 (Wang et

al. 2003), from dysfunction of the enzyme glutamine synthetase, an enzyme that metabolizes glutamate into glutamine as a neuroprotective function (Visalli et al. 2007) and from glutamate released by mononuclear phagocytic cells (Erdmann et al. 2009).

However, seemingly conflicting findings of low concentrations of Glu have been reported by several groups using 1D-MRS in both impaired and cognitively normal HIV patients (Mohamed et al. 2010; Sailasuta et al. 2009; Ernst et al. 2010) compared to our 2D-MRS results. In TE-averaged PRESS, TE was incremented from 35 ms to 190 ms and 1D spectra were averaged over 32 TE values resulting in severe T2-weighting (Ernst et al. 2010). In our case, signals with 100 t_1 increments with several TEs (30 ms to 79 ms) were acquired and without adding these signals, they were used to frequency label different metabolite resonances along the 2nd dimension. These two methodologies display different characteristics after Fourier transformation and the outcomes need to be interpreted cautiously.

Since our study with 2D-MRS used a different technology, examined a much larger voxel (27 mL) which included both frontal white and gray matter, and evaluated a group of youth exposed to HIV when their brains were still developing, different results might be expected. A recent study of adults with primary HIV infection found increased Glu/Cr in parietal grey matter. (Young et al. 2012).

Although Scy or Glu metabolite ratios did not correlate with neurocognitive performance, we did demonstrate that the healthy controls with faster attention/processing speed had lower NAA/tCr which could reflect that better performance was associated with a higher metabolism (with a higher total creatine). This inverse relationship between the lower NAA/tCr and faster attention/processing speed was not observed in the HIV-infected youth since their attention/processing speed was significantly slower and might have been altered by other brain injury mechanisms.

The limitations of our study include the restriction of our neurocognitive assessments to only three study domains of cognition, attention/processing speed, psychomotor function, and executive/problem solving skills. A broader assessment of cognitive ability may have revealed more group differences and associations with cerebral metabolites. In addition, we limited the 2D-MRS study to the right frontal brain due to time constraints. 2D metabolites in the left frontal brain or other brain regions may correlate better with various domains of cognitive ability (e.g., memory encoding) (Wright et al. 2011). Also the lack of group differences in the psychomotor and executive function may be due to the small sample size.

In summary, we found poorer attention/processing speed performances in the perinatally HIV-infected subjects relative to age matched HIV– control subjects. Our results in addition to the work of others support the importance of assessing attention/processing speed in this population. Finding a reliable, noninvasive assessment of brain health is critical for the long term evaluation of HIV-infected children and adults. Early detection of brain injury, either due to ongoing viral replication or excess neuroinflammation could potentially lead to a change in antiretroviral medications with better brain penetration, less neurotoxicity, and improved outcome. However, the best noninvasive tool for assessing brain health in this population remains to be determined. A recent study that compared three different combination antiretroviral treatment regimens over 48 weeks in adult patients (Winston et al. 2010) found that those with the most improvement in metabolite ratio (NAA/Cr) did not show the most neurocognitive improvement. Therefore, both spectroscopy and neurocognitive testing may be complementary in assessing central nervous system efficacy of antiretroviral therapies.

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Fig. 1.
Neurocognitive domain *T*-scores

Fig. 2.
2D L-COSY spectra of (a) Healthy (b) HIV Subjects

Table 1

Demographics for HIV+ and HIV– subjects

	HIV– Subjects (<i>n</i>=14) Mean (SD)	HIV+ Subjects (<i>n</i> = 16) Mean (SD)
Age	16.3 (2.3 years)	17.0 (2.9) years
CD4 count	N/A	536 (340)
%CD4>500	N/A	62.5 (<i>n</i> =10)
%CD4 >200	N/A	81.2 (<i>n</i> =13)
Log viral load	N/A	4.7 (1.3)
% Log Viral Load <1.9	N/A	56 (<i>n</i> =9)
% Log Viral Load>2.6	N/A	6.2 (<i>n</i> =1)
Age at first HIV treatment	N/A	4.6 (4.8) years
% treated at less than 1 year	N/A	25 (<i>n</i> =4)
% Male	64.3 (<i>n</i> =9)	50.0 (<i>n</i> =8)
% Known maternal substance abuse	N/A	25 (<i>n</i> =4)
% Hispanic	85.7 (<i>n</i> =12)	68.8 (<i>n</i> =11)
% African American	7.1 (<i>n</i> =1)	25 (<i>n</i> =4)
% African American/Hispanic	7.1 (<i>n</i> =1)	6.3 (<i>n</i> =1)

NA Not available or not applicable

Table 2

T-scores for three cognitive domains for HIV – and HIV+ youths

Cognitive Domains	HIV–(n=14) Mean(SD)	HIV+ (n=16) Mean(SD)	t	Cohen's d	p value
Attention/Processing Speed	50.87 (4.72)	45.72* (5.54)	2.715	1.03	0.011*
Psychomotor	48.25 (6.57)	45.63 (6.11)	1.132	0.43	0.267
Executive/Problem solving	47.41 (5.27)	45.07 (5.85)	1.143	0.43	0.263

Higher T-score indicates better performance

* p<0.05

Table 3
Right frontal lobe cerebral metabolite ratios for HIV – controls and HIV + patients

Metabolites	Metabolites/tCr		Metabolites/ tCho		p value
	Controls Mean±SD	Patients Mean±SD	Controls Mean±SD	Patients Mean±SD	
NAA	1.30±0.18	1.38±0.18	2.59±0.65	2.75±0.39	0.486
Asp *	0.43±0.10	0.51±0.08	0.86±0.25	1.01±0.20	0.092
GABA	0.27±0.18	0.20±0.16	0.52±0.39	0.42±0.33	0.580
Gln	0.06±0.05	0.06±0.08	0.16±0.23	0.14±0.15	0.706
Glu *	1.67±0.22	1.88±0.22	3.26±0.63	3.76±0.60	0.048
GSH	0.34±0.13	0.28±0.15	0.66±0.34	0.56±0.30	0.361
mI	1.18±0.38	1.44±0.26	2.33±0.92	2.86±0.48	0.071
PE	0.48±0.12	0.52±0.14	0.99±0.26	1.02±0.19	0.065
Scy *	0.06±0.01	0.08±0.02	0.12±0.04	0.16±0.04	0.018
Cr			1.97±0.36	2.00±0.31	0.754
NAA+ NAAAG	1.33±0.13	1.38±0.17	2.63±0.57	2.76±0.39	0.534
PC+ PCh+ Cho	0.49±0.04	0.51±0.09			
Gln+ Glu *	1.74±0.23	1.94±0.26	3.40±0.70	3.88±0.64	0.082

* $p < 0.05$

$p < 0.05$ are bolded