Reduced brain N-acetylaspartate suggests neuronal loss in cognitively impaired human immunodeficiency virus-seropositive individuals: In vivo $^1$H magnetic resonance spectroscopic imaging

D.J. Meyerhoff, Dr.RerNat; S. MacKay, MD; L. Bachman, PsyD; N. Poole, MS; W.P. Dillon, MD; M.W. Weiner, MD; and G. Fein, PhD

**Article abstract**—We used magnetic resonance imaging (MRI) and water-suppressed proton magnetic resonance spectroscopic imaging to study the effects of human immunodeficiency virus (HIV) infection on the brains of 10 individuals with cognitive impairment due to HIV and seven normal controls. $^1$H spectra from nine 2.5-ml volumes in the centrum semiovale and the mesial cortex showed significantly reduced N-acetylaspartate (NAA) relative to choline and creatine in the cognitively impaired HIV-infected subjects. This reduction was due to a nonlocalized decrease of NAA in these patients, only two of whom had moderate atrophy and white matter signal hyperintensities on MRI. Since NAA is a putative neuronal marker, the findings suggest neuronal damage in early stages of HIV infection that is not evident on standard MRI and are consistent with the neuropathologically known neuronal loss.

N. Vickers
trum of the normal human brain is from compounds containing an acetyl moiety bound to a nitrogen atom (N-acetyl). The primary contributor to this resonance is the amino acid N-acetylaspartate (NAA). Evidence suggests that it is only found in neurons and not in mature glial cells and in the cortex, NAA is located in neuronal cell bodies, whereas in the white matter it is located largely in axons. \(^{18,19}\) \(^1\)H MRS also measures creatine (Cr) (as a sum of phosphocreatine and creatine) involved in cellular energy metabolism and choline (Cho), a molecular moiety found in compounds involved in biomembrane metabolism.

In this study, we used both MRI and \(^1\)H magnetic resonance spectroscopic imaging (\(^1\)H MRSI) of a 17-mm-thick section through the supraventricular cerebrum to test the hypothesis that NAA is reduced in HIV-infected individuals with cognitive impairment. Such a finding may indicate a loss of neuronal density or viability, or both. Furthermore, standard MR images were analyzed for atrophy to test the hypothesis that loss of NAA in cognitively impaired HIV-seropositive (CISP) individuals is a more sensitive marker of HIV effects than atrophy.

**Methods. Subjects.** All subjects gave prior informed consent (approved by the Committee on Human Research at UCSF). Fourteen HIV-seropositive men (mean age, 38 ± 9 years (±SD)) were recruited from the HIV clinic and via flyers distributed with hot meals to impaired HIV-seropositive patients in the community. At the time of the study, four of the HIV-seropositive subjects were still working, four had an AIDS-related–complex diagnosis, and none had yet developed opportunistic infections. Twelve subjects complained of significant memory loss, with 10 reporting medical problems including fatigue, weight loss, balance difficulties, diarrhea, thrush, headaches, swollen glands or lymph nodes, shigellosis, lymphoma, and hairy leukoplakia. Given this clinical picture, the HIV-seropositive individuals were distributed among CDC stages II, III, IV A, and IV B. Controls were healthy HIV-seronegative volunteers (five men, two women; mean age, 33 ± 10 years). Both groups were screened to exclude individuals with current or past history of medical, neurologic, or psychiatric disorder or alcohol or substance abuse, except for current medical and neuropsychiatric problems clearly secondary to the HIV infection.

**Neuropsychological assessments.** Subjects were administered 14 neuropsychological tests measuring a wide range of cognitive skills, including attention, concentration, memory retention, verbal language, problem-solving, visuomotor and visuospatial skills, and fine motor ability: WAIS-R Digit Span, WAIS-R Digit Symbol, Shipley Institute of Living Scale, finger tapping, grip strength, Rey-Osterrieth Complex Figure, Luria 99, Wechsler Logical Memory (Immediate and Delayed), Wechsler Visual Reproduction (Immediate and Delayed), Fuld Object-Memory Evaluation-15,20 short categories test, Controlled Oral Word Association Test, and Trails A and B. Each test was rated for presence and severity of impairment on a 0- to 2-scale and the impairment scores were summed. The degree of overall impairment on the summed scores was then classified as follows: 0 to 1 = no impairment, 2 to 5 = mild impairment, 6 to 9 = moderate impairment, and 10 or more = severe impairment. The groups’ degrees of impairment were as follows: four subjects had no impairment (asymptomatic HIV-seropositive [ASP]) (mean age, 40 ± 12 years); five subjects had mild impairment; three subjects had moderate impairment; and two subjects had severe impairment. Mean age of the 10 impaired (CISP) subjects was 37 ± 6 years. Given the relatively high socioeconomic and educational levels of the Bay Area homosexual population, this assessment and screening scheme was conservative, tending to underestimate the degree of cognitive impairment.

**Magnetic resonance.** All MR studies were performed on a whole-body, 2-tesla MRI/MRS system (Phillips Medical Systems, Shelton, CT). The procedures for MRI and \(^1\)H MRSI \(^{21,22}\) were the same as previously described, except for the following modifications: the sections for transverse MRI were angulated along the canthomeatal line. Figure 1 shows a midline sagittal MR image of a normal volunteer with the angulated transverse MRI sections and the MRSI region. Sixteen to 24 contiguous sections of 5.62-mm thickness (TR/TE = 2,500 msec/30 msec, 80 msec) were obtained to cover the entire brain from cebellum to vertex. MR images were evaluated by a board-certified neuroradiologist blind to each subject’s serostatus. Ventricular and sulcal atrophy were rated separately as absent, mild, moderate, or severe. WMSIs were rated on a 0- to 4-scale previously published. \(^{23}\) After MRI, a 17-mm-thick volume of interest (VOI), corresponding in location and thickness to three MCI voxels, was selected for \(^1\)H MRSI. Each test was rated for presence and seventy of scoring by a board-certified neuroradiologist blind to each subject’s serostatus. Ventricular and sulcal atrophy were rated separately as absent, mild, moderate, or severe. WMSIs were rated on a 0- to 4-scale previously published. After MRI, a 17-mm-thick volume of interest (VOI), corresponding in location and thickness to three MCI voxels, was selected for \(^1\)H MRSI. The VOI was generally chosen to include the very top of the corpus callosum and the two superior cranial MRI sections. The anterior-posterior and left-right dimensions of the VOI were adjusted for every subject according to brain size (generally, approximately 100 mm [anterior-posterior] by 90 mm [left-right]). The position and angulation of a typical VOI is depicted in figure 1, A and B. The parameters selected for \(^1\)H MRSI resulted in a nominal in-plane resolution of 11 mm and a nominal MRI volume element (voxel) size of approximately 2.2 ml. Total acquisition time was 34 minutes; the entire MRSI and MRI examination took less than 2 hours.

**Data processing.** The MRSI data and transverse MR images were further analyzed using home-written spectroscopic imaging display software. The MRSI spectral dimension was zero-filled to 1,024 points; both spatial dimensions were zero-filled to 32 points. A 1-Hz exponential line broadening was applied in the time domain. For both spatial domains, a mild gaussian multiplication was used, corresponding to a broadening of 1 mm and resulting in a final effective voxel size of approximately 2.5 mm. After Fourier transformation in spectral and spatial dimensions, two-dimensional MRS images were published by integration over selected spectral regions. For spatial selection of the spectra to be analyzed, the spatially correlated summed MR images (composed of three thin MCI sections) was used exclusively. Spectra were extracted from nine voxels within the VOI overlaid on the MRT image. The location and size of the analyzed voxels are indicated on the transverse MRI shown in figure 1B. Voxels were selected in the following way: three voxels from the midline area of the brain (one from anterior mesial cortex, one from posterior mesial cortex, and one from an intermediate region) and three lateral voxels from each hemisphere in the frontal, anterior-parietal, and posterior-parietal regions. The three midline voxels were selected...
so that they contained as much gray matter (appearing bright on T₂-weighted MR images) as possible, avoiding white matter tissue. The six lateral voxels were selected so that they contained a maximum of white matter tissue (appearing dark on the T₂-weighted MR image), avoiding large sulci. The nine extracted magnitude spectra were fitted with gaussian shapes. Peak areas were derived from the NMRl software (New Methods Research, Syracuse, NY) for automated line-fitting and peak area determination. Following manual setting of the baseline midway through the noise, three gaussian peaks were fitted to the three major resonances in the spectra (Cho, Cr, and primarily NAA). The peaks were fitted with gaussian rather than lorentzian lines because they are due to multiple compounds, and because spectral residuals after line-fitting were smaller with fitting gaussian line-shapes. Peak areas were derived from the NMRl software in arbitrary units; no intensity standard was included in the studies. Since absolute peak areas are affected by possible long-term spectrometer instabilities, peak area ratios were used for primary data analysis.

Statistical analysis. Repeated measures of analysis of variances (ANOVA) were used to compare metabolite ratios and metabolite peak areas by location and groups. All values are expressed as mean ± 1 SD, and p < 0.05 was considered statistically significant.

Results. On transverse T₂-weighted MR images, three of 14 (21%) HIV-seropositive subjects (two of whom were cognitively impaired) showed moderate periventricular widening. One of these impaired individuals also evidenced large regions of periventricular WMSHs in the centrum semiovale (grade 4 on our rating scale). Eight of ten CISP subjects and three of four cognitively ASP individuals had normal MR images.

Figure 2 shows typical ¹H MRSI spectra obtained from the nine analyzed voxels for a moderately impaired HIV-seropositive patient with normal MRI. Three major resonances due to (1) N-acetyl-containing compounds (primarily NAA), (2) creatine and phosphocreatine (Cr), and (3) Cho-containing compounds are visible in all spectra. Slight variations in signal height, particularly of the NAA and Cho resonances, are discernible.

Mean metabolite ratios were compared among the three groups for all locations. Reduced NAA/Cho and NAA/Cr ratios were observed in CISP patients versus normal controls across all nine voxels, with no significant group by location interactions when comparing lateral voxels with midline voxels or when comparing anterior with posterior voxels. Mean NAA/Cho was reduced by 14%, from 2.28 ± 0.16 in controls to 1.98 ± 0.21 in CISP subjects (F(1,14) = 9.60; p = 0.008). Mean NAA/Cr was also reduced by 14%, from 3.23 ± 0.46 in controls to 2.81 ± 0.34 in the CISP group (F(1,14) = 4.75; p = 0.047). No significant differences were found between CISP and ASP groups or between ASP and control groups.

Figure 3 displays NAA/Cho and NAA/Cr for the CISP subjects and HIV-seronegative controls. Since no significant regional differences were observed, ratios from the nine voxels of each subject were aver-
The reduced NAA in patients compared to controls (1.43 ± 0.23 in the control group) further suggests a reduced NAA in the brain. The absolute metabolite analysis of integrals involving significant differences were not brain regions. In the analysis of integrals involving brain significant differences, there was a trend toward reduced Cr (4.04; t₁₅ = 1.94). In this patient group, from those incorporating integrals subjects versus effects of group, NAA/Cr ratios were significantly reduced in HIV-seropositive (CISP) subjects. The increased NAA/Cr ratios obtained from CISP individuals as compared to asymptomatic HIV-seronegative controls, asymptomatic HIV-seropositive (ASP) individuals and cognitively impaired HIV-seropositive (CISP) individuals. The significant difference was observed, as were averaged over all nine voxels, and each data point represents an average ratio. o = individuals with normal MRI; + = individuals with moderate atrophy and moderate periventricular white matter signal hyperintensities on MRI; * = individuals with moderate atrophy and white matter signal hyperintensities on MRI.

Discussion. Significant differences were observed between the NAA/Cho ratio and the NAA/Cr ratio in CISP individuals and normal controls. The data are consistent with the presence of cortical and subcortical atrophy in HIV-seropositive patients. The imaging technique allowed examination of the hippocampus and the subcortical white matter, which is often affected in HIV-seropositive patients.

Figure 2. Nine 1H MRSI spectra obtained from the nine voxels indicated on the MR image of figure 1B. (Left) Spectra from MR image left; (right) spectra from MR image right; (middle) spectra from midline voxels of the MR image. Resonances are from NAA at 2.03 ppm, from Cr at 3.05 ppm, and from Cho at 3.25 ppm.

Figure 3. NAA/Cho (left) and NAA/Cr (right) in cognitively normal HIV-seronegative controls, asymptomatic HIV-seropositive (ASP) individuals, and cognitively impaired HIV-seropositive (CISP) individuals. Because no significant regional differences were observed, ratios were averaged over all nine voxels, and each data point represents an average ratio. o = individuals with normal MRI; + = individuals with moderate atrophy and moderate periventricular white matter signal hyperintensities on MRI; * = individuals with moderate atrophy and white matter signal hyperintensities on MRI.

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-aged and are represented by one data point per subject. Nine of ten CISP individuals had NAA/Cho and NAA/Cr below the mean of the normal controls, and four of 10 CISP subjects (one of whom displayed moderate atrophy and WMSHs on MRI) had NAA/Cho values outside the range of the normal controls.
The reduced NAA/Cho and NAA/Cr in CISP patients compared with HIV-seronegative controls, together with the virtually identical Cho/Cr in both groups (1.43 ± 0.10 in the CISP group versus 1.43 ± 0.23 in the controls), indicate an underlying difference involving NAA concentrations. To examine further the a priori hypothesis (see introduction) of reduced NAA in CISP individuals, an ANOVA of absolute metabolite integrals was performed. As in the analysis of ratios, all interactions of metabolite integrals involving group and location were nonsignificant (all p's < 0.25), indicating that group differences were not differentially present in specific brain regions. For NAA, but not for Cho or Cr, there was a trend toward reduced integrals in the cognitively impaired group (0.38 ± 0.04 versus 0.42 ± 0.04; t(15) = 1.94; p = 0.07), corresponding to a 10% reduction of the NAA signal integral.

NAA ratios and integrals for the four ASP patients in this study were not significantly different from those for controls or CISP subjects. When incorporated into the analysis of HIV-seropositive subjects versus HIV-seronegative controls, the effects of group differences of NAA/Cho and NAA/Cr were diluted. NAA/Cr was reduced by only 11%, from 3.23 ± 0.46 in controls to 2.89 ± 0.34 (n = 14) in HIV-seropositive patients (F[1,17] = 4.45; p = 0.05), while total NAA/Cr was reduced by only 9%, from 2.28 ± 0.16 in controls to 2.08 ± 0.30 in the HIV-seropositive group (F[1,17] = 2.35; p = 0.14). Also in this analysis, no specific regional differences were observed between groups. Figure 3 depicts metabolite ratios obtained for the four ASP subjects, demonstrating an overlap with metabolite ratios obtained from HIV-seronegative controls and CISP individuals.

Although the number of subjects studied was small, there were a strong (but insignificant) correlation between NAA/Cho and CD4 percent (r = 0.51; p = 0.074; n = 13 [one subject had no CD4 assessment]). However, this correlation was primarily due to extreme values of NAA/Cho for two patients. A very weak trend for a correlation of NAA/Cho with cognitive impairment (r = -0.29; p = 0.32) was observed. In addition, over all controls and HIV-infected individuals (n = 21), there was a significant negative correlation between age and NAA/Cr (r = -0.46; p = 0.035) and no correlation between age and NAA/Cho (r = -0.02).

Discussion. Significantly reduced ratios of NAA/Cho and NAA/Cr were found in a group of 10 CISP individuals in relatively early stages of the disease compared with age-matched HIV-seronegative controls. The results suggest nonlocalized neuronal damage in patients with cognitive impairment secondary to HIV infection. This report is the first demonstration of noninvasive imaging of a naturally occurring marker of neurons in the brain of HIV-seropositive patients.

The imaging nature of the spectroscopic technique allowed examination of a large section of the brain in situ, avoiding the usual focus of autopsy studies on small preselected brain regions. The data showed reduced NAA/Cho and NAA/Cr in the supraventricular brain slice studied, including midline voxels containing mesial cortex neurons, and voxels in both hemispheres containing predominantly white matter. Analysis of the absolute signal integrals showed that this difference resulted from a 10% reduction of the signal integral from NAA in the CISP group, with integrals for Cho and Cr virtually unchanged. The findings are consistent with an overall reduction in the number of neurons found in neuropathologic studies, which demonstrated cortical thinning in HIV-infected individuals. The results leave open the question of whether there is a real reduction of neuronal density/NAA concentration in cognitively asymptomatic HIV-seropositive patients.

Neuronal loss was recently reported in two HIV-seropositive patients with clinical but without MRI evidence of CNS involvement. 1H MRS spectra from two large volumes in the parietal lobe from an area that appeared normal on MR images showed reduced NAA/Cho and NAA/Cr relative to normal controls. In the current study, a larger patient group was examined and spectra were obtained from smaller voxels throughout a larger section of the brain. This enabled spatial mapping of NAA, suggesting nonlocalized neuronal damage secondary to HIV infection.

In our cohort of 10 CISP patients without opportunistic infections, 80% had normal T2-weighted MR images, while 20% showed atrophy and WMSHs, adding further evidence to the fact that cognitive impairment due to HIV infection is rarely manifest on MR images as abnormal anatomy. Due to the ability to measure the neuronal marker NAA, 1H MRSI may be specifically sensitive to neuronal damage. Ninety percent of the CISP patients had NAA/Cr below the normal means, and 40% had values outside the range of the normal controls. 1H MRSI thus seems more sensitive than T2-weighted spin-echo MRI in assessing early effects of HIV infection on the brain. It may become an objective measure of neuronal involvement in HIV disease as well as in other dementing illnesses, such as Alzheimer's disease.

The primary statistical analysis in this study was performed on metabolite ratios that are not affected by long-term instrumental instabilities. The secondary analysis of absolute metabolite integrals revealed a strong trend toward a reduction of the NAA signal integral in the CISP individuals relative to HIV-seronegative controls (p = 0.07), with no difference between groups in Cho and Cr integrals. Both analyses taken together suggest a selective replacement of neurons by glial cells not containing NAA. The ANOVA of NAA integrals underestimates the full HIV effect, since the precision of the estimate of the NAA effect is reduced because of long-term instrumental instability incor-
orporated into the error term.

Although the observed reduction of NAA signal intensity in the brains of CISP patients is consistent with neuropathologically known neuronal loss, alternative interpretations need to be considered. We do not believe that the results are due to selective changes in either $T_1$ or $T_2$ relaxation times of NAA in the brains of CISP individuals. Such scenarios would require changes in relaxation times specific to the NAA resonance, with Cho or Cr resonances, which come from the same voxel and tissue as NAA, being unaffected. The NAA resonance includes weak signals from other N-acetyl-containing metabolites. Changes in concentrations, relaxation times, or both, of compounds other than NAA could conceivably account for part or all of the observed reduction of NAA signal intensity secondary to HIV infection.

Interpretations other than frank neuronal loss include loss of dendritic arborization, vacuolation or degeneration of axons, or both, and a reduction of NAA concentration within intact neurons. In this regard, presynaptic terminal loss in frontal cortex and degeneration of axons were reported in patients with AIDS dementia complex. Because of its inherently low spatial resolution, MRS is only sensitive to the amount of signal-producing tissue rather than its structure or fine pattern on a cellular level. Our preliminary findings, however, seem to suggest that there is no differential loss of NAA between midline voxels of the mesial cortex and lateral volumes in white matter tissue, indicating that neuronal cell bodies and axons may be affected to approximately the same extent.

For the prognosis and treatment of HIV infection of the brain, it is of critical importance to distinguish between loss of neurons and decrease in the number and size of neuronal structures. While lost neurons are not replaceable, reARBoration of dendritiic structures may be possible if the morbidity process is arrested. In this regard, it would be of interest to know how NAA concentrations parallel the amelioration of HIV-related cognitive impairment secondary to zidovudine treatment. To optimize treatment effectiveness, treatment should be initiated as early in the disease process as possible. This requires early detection of brain involvement. If this proves possible with 1H MRSI, and if it can detect degeneration of neuronal structures before irreversible neuronal loss occurs, treatment could be initiated to arrest or reverse neuronal dysfunction, with 1H MRSI employed to monitor outcome.

In conclusion, 1H MRSI appears to be promising as a new neuroimaging modality more sensitive than spin-echo MRI to early brain effects of HIV infection. Significantly reduced NAA relative to Cho and Cr was found in CISP individuals with and without atrophic changes and WMSHs on MR images. These results suggest neuronal damage in HIV infection and are consistent with neuropathologic findings of neuronal loss. In vivo imaging of neurons may be helpful in quantifying the state and progression of brain involvement in HIV infection and other neurodegenerative diseases.

**References**