Elevated subcortical choline metabolites in cognitively and clinically asymptomatic HIV+ patients

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Article abstract—Objective: To determine whether the concentrations of the neuronal marker N-acetylaspartate (NAA) and the choline-containing metabolites (Cho) are altered in the subcortical brain of HIV+ patients who are cognitively normal and clinically asymptomatic, and to determine whether these alterations are greater in the presence of cognitive impairments and clinical symptoms. Background: Pathologic studies suggest that subcortical gray matter carries a heavy HIV load, and neuropsychological test results are consistent with involvement of subcortical and frontostriatal brain systems in HIV disease. Noninvasive proton magnetic resonance spectroscopy (1H MRS) suggests neuronal preservation and macrophage infiltration in the subcortical brain of clinically symptomatic and cognitively impaired HIV+ individuals. Improved 1H MRS methods may allow the early detection of metabolite alterations in the subcortical brain of asymptomatic HIV+ individuals. Methods: Two-dimensional 1H MRS imaging was performed on 30 HIV- control subjects and 70 HIV+ patients with varying severities of systemic disease and neuropsychological impairments, but without cerebral opportunistic infections. Results: Subcortical Cho was elevated in HIV+ patients compared with control subjects regardless of the presence or absence of cognitive impairment or clinical symptoms. Subcortical NAA was lower than control NAA only in severely cognitively impaired HIV Subjects. Subcortical NAA correlated with performance on a variety of neuropsychological tests but not with Centers for Disease Control clinical stage, whereas high-thalamic Cho was associated with low CD4 lymphocyte counts. Conclusions: 1H MRS imaging detects higher Cho in subcortical brain early in HIV disease, when individuals are clinically and neuropsychologically asymptomatic, whereas lower NAA is only found in subcortical brain in individuals with severe neuropsychological impairments. Quantitative 1H MRS imaging may play a role in the objective assessment of the presence, magnitude, and progression of brain involvement in HIV infection.

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In a study of 389 men infected with HIV type 1, Heaton et al. found increasing rates and severity of cognitive impairment at successive stages of HIV infection. HIV-related neuropsychological impairment was generally mild, especially in the earliest, medically asymptomatic stage of infection. The cognitive abilities most often affected in HIV disease were attention, speed of information processing (psychomotor performance), and learning efficiency, a pattern consistent with involvement of subcortical, frontostriatal, and frontolimbic brain systems. Other studies have shown that HIV invades the brain early during the course of infection, with subcortical gray matter structures carrying some of the heaviest viral loads in the brain, in addition to infection-associated increases in microglia, lymphocytes. It has been suggested that this subcortical neuropathology has an important role in the development of HIV-associated dementia. In a preliminary proton magnetic resonance spectroscopic imaging (1H MRS) study of subcortical brain in eight clinically symptomatic and cognitively impaired HIV+ subjects compared with eight cognitively normal HIV- control subjects, we found metabolite ratios that suggested higher levels of choline-containing metabolites (Cho) and unchanged levels of N-acetylaspartate (NAA), a neuronal marker. These findings were consistent with subcortical brain histopathologic findings in HIV disease of neuronal preservation (consistent with unchanged NAA) and macrophage infiltration or gliosis (consistent with high Cho). Cognitively normal HIV+ individuals in the early stages of the disease were not part of that preliminary 1H MRS study. Other recent studies suggest that 1H MRS metabolite alterations, including those in subcortical gray matter structures, can be detected in asymptomatic HIV+ patients.

In the current study we investigated whether our initial observations could be replicated in a much...
larger sample of cognitively impaired HIV+ patients, whether they extend to cognitively normal HIV+ patients, and whether metabolite measures are associated with measures of the severity of brain dysfunction or with measures of the severity of systemic disease. Compared with our earlier study, the current study used an expanded neuropsychological test battery, a newer generation of MR scanner, and methods that allow assessment of absolute metabolite signal integrals, rather than being limited to analysis of metabolite ratios. We posed the following three hypotheses:

1. Compared with healthy HIV− control subjects, Cho levels are elevated and NAA levels are unchanged in the subcortical brain of cognitively impaired HIV+ patients.

2. Compared with healthy HIV− control subjects, subcortical Cho levels are elevated in cognitively normal HIV+ patients.

3. The magnitude of subcortical metabolite changes is associated with the severity of neuropsychological impairments.

Subjects and methods. Subjects. A total of 100 subjects participated in this cross-sectional study (table 1). All subjects’ clinical status and blood work (complete blood count profile, differential count, and lymphocyte subset, including CD4 rates and percentiles in HIV+ subjects, and ELISA test for HIV antibodies in control subjects) were evaluated within 3 weeks of the MRI/MRS examination. Centers for Disease Control (CDC) clinical status (modified to exclude neurocognitive indicators) of the 70 HIV+ patients (69 men, 1 woman) was as follows: 7 were asymptomatic (CDC A), 36 were symptomatic with symptoms other than cognitive impairments (CDC B), and 27 had AIDS indicator conditions other than HIV dementia (CDC C). Fifty-nine HIV+ patients (84%) were severely immunosuppressed with T-helper cell counts < 200 cells/μL; the rest of the patients had T-helper cell counts between 200 and 499 cells/μL. The HIV+ patients were compared with 16 comparably aged HIV− high-risk control subjects (15 men, 1 woman, including both homosexual and bisexual individuals) and 14 HIV− low-risk control subjects (13 men, 1 woman, including only heterosexual individuals).

Participants were recruited from advertisements in local newspapers, from local HIV medical and social service clinics, and via flyers distributed with hot meals to HIV+ patients in the community. The control subjects were studied interspersed with the HIV+ patients, and the MRI/MRS system operators and spectral analysts were blinded to subjects’ HIV and clinical status. All studies were performed between the spring of 1994 and the fall of 1996, when potent combination antiretroviral treatments were not available except in research settings.

Potential subjects were screened to exclude individuals with a current or past history of medical, neurologic, or psychiatric disorders, or alcohol or substance abuse, except for current medical and neuropsychiatric problems clearly secondary to HIV infection. Subjects were excluded for a history of head injury with loss of consciousness, stroke, cortical or subcortical infarctions, or other major abnormalities evidenced on MR images as assessed by a neuroradiologist (D.N.). Subjects with opportunistic infections of the brain (either diagnosed by their AIDS doctor or evident on their MRI examination) were excluded. The local committee on human research approved the protocol, and all subjects (or their legal guardians in three instances) gave written informed consent before participating in the study.

Neuropsychological testing. All subjects were administered the computerized MicroCog Assessment of Cognitive Functioning (standard version), which includes 18 subtests that are used to assess performance in the attention, abstraction, spatial processing, memory, and reaction time domains. A trained psychometrician supplemented the MicroCog Assessment with a number of other tests including the Beck Depression Inventory (short form), the Shipley Institute of Living Scale, the Rey-Osterreith Complex figure (immediate and delayed recall), the Trail-Making Test A and B, the Symbol Digit Modalities Test (written and oral), the Controlled Oral Word Association Test (COWAT), the Grooved Pegboard Test, the Short Category Test (booklet format), and the Stroop Color and Word Test. The MicroCog assessment took approximately 45 to 60 minutes to complete, and the expanded battery added 1 hour of testing.

Age- and education-adjusted z-scores were calculated for all tests (including the MicroCog subtests) and were based on each test’s normative data from the references.
cited earlier. The z-scores were averaged for cognitive domains as follows: 1) attention (numbers forward, numbers reversed, MicroCog alphabet, MicroCog word list 1), 2) verbal (COWAT, Shipley vocabulary tests), 3) abstraction (Shipley abstract score, short categories, Stroop interference score, Trail-Making Test B, MicroCog analogies, MicroCog object match A and B), 4) spatial processing (MicroCog tic tac, MicroCog clocks), 5) psychomotor (Trails A, oral and written symbol digit), 6) immediate memory (MicroCog story immediate 1 and 2, Rey immediate, MicroCog word list 2), 7) delayed memory (MicroCog story delay 1 & 2, MicroCog address delay, Rey delay recall), 8) motor (Grooved Pegboard Test), and 9) reaction time (MicroCog timers 1 and 2).

Computation of a global clinical impairment score (GCIS) and a mean impairment z-score. Each domain’s average z-score was referred to the cumulative normal distribution and then converted to a percentile score and finally to a clinical impairment score. A clinical impairment score of 0 was assigned to domain z-scores falling above the 95th percentile, a clinical impairment score of 1 was assigned to domain z-scores falling at or below the 95th and above the 90th percentile, and a clinical impairment score of 2 was assigned to domain z-scores falling at or below the 5th percentile. The domain clinical impairment scores (0, 1, or 2) were then summed across domains to yield a GCIS. The GCIS was a relatively conservative assessment of impairment, in that subjects would accrue a 1 only if they performed below the 15th percentile in a cognitive domain. Subjects with a GCIS of 0 or 1 were classified as cognitively normal (CN). Subjects with a GCIS of 2 or greater showed evidence of clinical neuropsychological impairment in at least one cognitive domain, and were classified as having some clinical cognitive impairment. Subjects with a GCIS of 2 to 5 were considered mildly to moderately cognitively impaired (mCI), whereas those with a GCIS of 6 or more were considered severely cognitively impaired (sCI). To facilitate examination of correlations between MRS variables and global neuropsychological performance, we computed the average age- and education-standardized z-score across all neuropsychological domains (i.e., the mean impairment z-score). This measure, in comparison with the GCIS, is of a finer grade and is an integer scale variable that does not have a floor (e.g., a GCIS of 0).

Patient classification. All HIV+ patients were classified into cognitive impairment groups without regard for CDC clinical status, and were classified according to systemic disease into modified CDC clinical categories without regard for their cognitive impairment status (see table 1). Cognitively normal HIV+ patients were not different from control subjects in GCIS, but evidenced systemic disease and cognitive impairment than control and cognitively normal HIV+ subjects.

MRI/MRSI examinations. All studies were performed on a 1.5-T Magnetom VISION system (Siemens Inc., Iselin, NJ) equipped with a standard quadrature head coil. A padded head holder was used to restrict subjects’ head movements. The MRI protocol consisted of sagittal T1-weighted localizer scans, oblique axial double spin-echo (SE) scans angulated along a line drawn at −10 deg from the planum sphenoidale as seen in the midsagittal localizer image, and a volumetric (three-dimensional [3D]) magnetization prepared rapid gradient echo (MP-RAGE) acquisition angulated perpendicular to the optic nerve as seen in the midsagittal localizer image. The measurement parameters for double SE were repetition time [TR]/echo time [TE], 1,500/30/50 msec; 1.0 × 1.0-mm in-plane resolution; and 49 contiguous 3-mm-thick slices covering the entire brain from the inferior cerebellum to the vertex. The measurement parameters for 3D MP-RAGE were TR/ inversion time [TI]/TE, 10/250/4 msec; flip angle, 15 deg; resolution, 1.0 × 1.0 mm; and 1.4-mm–thick partitions. Total imaging time was less than 45 minutes. All MR images were read by a neuroradiologist for ventricular and sulcal atrophy, for abnormal signal intensities in the white matter, and for other abnormalities.

3H MRSI data sets were acquired using a SE two-dimensional MRSI sequence at TR/TE 1,800/135 msec, with preselection of a volume of interest (VOI) by point-resolved spectroscopy (PRESS). Before data acquisition the magnet was shimmed manually on the VOI selected for PRESS 3H MRSI. Typical water line widths at half height were between 5 and 10 Hz. Three PRESS VOIs were acquired in each subject: a VOI through the subcortical brain, a VOI through the ventricles, and a VOI placed above the ventricles. Results from the subcortical VOI are the topic of this article. All PRESS VOIs were selected from midsagittal and axial SE MRIs, were angulated parallel to the double SE image plane, and were positioned such that five 3-mm–thick double SE sections corresponded exactly to the nominal MRSI slice thickness of 15 mm. The subcortical VOI was centered on the thalamus and intersected the head of the caudate and the lenticular nuclei. The anterior–posterior and left–right dimensions of the VOIs were adjusted on every subject according to brain size, with typical dimensions of 120 mm anterior–posterior and 70 mm left–right. A representative angulation and position of the subcortical PRESS VOI is depicted in figure 1. The MRSI field of view was 210 × 210 mm, and was sampled using a circular k-space scheme equivalent to 24 × 24 phase encoding steps, resulting in a nominal spatial MRSI resolution of 1.1 mL. The spectral sweep width was 1,000 Hz, acquisition size was 512 words, and acquisition time was 13 minutes per 3H MRSI study.

MRS analysis. The 3H MRSI data were zero filled on a SUN (Mountain View, CA) workstation to a rectangular matrix of 32 × 32 × 1,024 points, Fourier transformed, and phase corrected using software developed in-house. Four-Hertz Gaussian line broadening was used in the spectral direction, and mild Gaussian apodization was applied along the spatial directions to reduce Gibbs ringing effects, resulting in an effective spatial MRSI resolution of less than 1.5 mL. An image summing the three axial T2-weighted MR images corresponding to the top, middle, and bottom of the MRSI section was used as a guide for voxel placement. Size and typical location of the selected subcortical MRSI voxels studied are indicated on the MR images of figure 1. Six single-voxel spectra were extracted, two each from the bilateral caudate nuclei, the lenticular nuclei, and the thalami. At the time of voxel selection, the operator was blinded to the quality of the spectra. There was little allowance for variation of voxel location in the caudate nucleus due to the size of the structure; the se-
The axial MR image (TR, 2,575 msec; TE, 80 msec) is the center slice of a stack of five 3-mm-thick sections that correspond exactly to the thickness of the VOI used for MRS imaging.

Selected voxels had to include at least 50% of the caudate as seen on the summed MRI (this was accomplished for all subjects). Lenticular voxels were placed on the midline between globus pallidus and putamen as seen on the summed MRI to include as much lenticular tissue as possible. In more than 90% of the subjects these voxels included only the lenticular nucleus as visualized on the summed MRI. Thalamic voxels were placed in the center of the thalami as seen on the summed MRI and included thalamic tissue only.

After application of a linear baseline correction routine when necessary, spectra were curve fit using NMR1 software (version 1.4.5., New Methods Research Inc., Syracuse, NY) to yield peak integrals for NAA, Cho, and creatine-containing metabolites (Cr). Figure 2 shows typical spectra obtained from an HIV+ CN individual. Selected spectra were excluded from data analysis when the signal-to-noise ratio of the NAA signal was less than 4 and spectral line widths exceeded 13 Hz, in which case Cho and Cr resonances could not be distinguished reliably on the frequency scale. Almost 50% of the caudate spectra had to be excluded from data analysis whereas almost all other extracted spectra were included in the data analysis (see Results). For further analysis, peak integrals were corrected for occasional receiver gain differences and coil loading (using the transmitter reference amplitude of a 180-deg pulse calculated by the MR scanner), allowing comparison between subjects. There was no subcortical atrophy (i.e., increased CSF spaces) obvious to the neuroradiologist reading the MR images. Nevertheless, we used the Cr integral as a covariate for NAA and Cho measures to correct for possible subtle atrophy in the voxel. This is based on the assumption that Cr is unchanged by the disease process and that any Cr loss reflects atrophy.

Statistical analysis. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC) and BMDP 5V (BMDP Statistical Software Inc., Los Angeles, CA). Analysis of variance (ANOVA) was used to test for differences in the age distribution, CD4 counts, GCIS, and mean neurocognitive test z-scores between groups. Metabolite integrals were tested for differences by group and location (thalamic, lenticular, and caudate nuclei) using repeated-measures ANOVA with covariates that change across repeated measures (BMDP 5V). This analysis used the Cr integral at each location as a covariate for the NAA and Cho integrals at the location. Brain Cr has been shown to be unchanged in many pathologies and was used here to correct for possible effects of atrophy in the spectroscopy voxels. In repeated-measures ANOVA, to determine the proper correlation structure for use in the analysis, we first performed an analysis assuming compound symmetry of the covariance matrix Σ, and then re-
Table 2 Subcortical metabolite measures (mean of thalami, lenticular, and caudate nuclei in arbitrary units) classified by GCIS

<table>
<thead>
<tr>
<th>Group</th>
<th>NAA, mean ± SD</th>
<th>Cho, mean ± SD</th>
<th>Cr, mean ± SD</th>
<th>Cho/Cr, mean ± SD</th>
<th>NAA/Cho, mean ± SD</th>
<th>NAA/Cr, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV- low risk</td>
<td>46.3 ± 4.0</td>
<td>29.0 ± 4.1</td>
<td>26.3 ± 4.2</td>
<td>1.21 ± 0.3</td>
<td>1.67 ± 0.24</td>
<td>1.87 ± 0.30</td>
</tr>
<tr>
<td>HIV- high risk</td>
<td>44.6 ± 7.8</td>
<td>26.6 ± 5.3</td>
<td>24.0 ± 5.2</td>
<td>1.20 ± 0.3</td>
<td>1.81 ± 0.42</td>
<td>1.94 ± 0.28</td>
</tr>
<tr>
<td>HIV- pooled</td>
<td>45.5 ± 6.0</td>
<td>27.9 ± 4.8</td>
<td>25.3 ± 4.7</td>
<td>1.21 ± 0.3</td>
<td>1.74 ± 0.34</td>
<td>1.90 ± 0.29</td>
</tr>
<tr>
<td>HIV+ CN</td>
<td>45.6 ± 5.0</td>
<td>31.4 ± 5.5</td>
<td>24.9 ± 2.9</td>
<td>1.35 ± 0.3</td>
<td>1.53 ± 0.28</td>
<td>1.88 ± 0.23</td>
</tr>
<tr>
<td>HIV+ mCI</td>
<td>43.5 ± 7.0</td>
<td>30.6 ± 5.4</td>
<td>25.1 ± 5.2</td>
<td>1.36 ± 0.4</td>
<td>1.49 ± 0.20</td>
<td>1.84 ± 0.40</td>
</tr>
<tr>
<td>HIV+ sCI</td>
<td>36.5 ± 6.9†</td>
<td>30.2 ± 9.5</td>
<td>22.2 ± 5.3</td>
<td>1.55 ± 0.5</td>
<td>1.34 ± 0.32</td>
<td>1.79 ± 0.30</td>
</tr>
<tr>
<td>HIV+ pooled</td>
<td>43.3 ± 6.8</td>
<td>30.9 ± 6.1*</td>
<td>24.6 ± 4.4</td>
<td>1.39 ± 0.39</td>
<td>1.48 ± 0.27</td>
<td>1.85 ± 0.32</td>
</tr>
</tbody>
</table>

Statistical comparisons of HIV+ subgroups with pooled control subjects were only performed if there was a significant difference among HIV+ subgroups.

* p < 0.01.
† p < 0.0001. All p values are relative to pooled HIV− control subjects, and are for Cr− corrected integrals.

GCIS = global clinical impairment score; NAA = N-acetylaspartate; Cho = choline-containing metabolites; Cr = creatine-containing metabolites; CN = cognitively normal; mCI = mild to moderate cognitive impairment; sCI = severe cognitive impairment.

peated the analysis allowing an arbitrary unstructured covariance matrix (i.e., with all variance and covariance parameters estimated from the data). A likelihood ratio test rejected the hypothesis that a has compound symmetry, and all subsequent analyses were performed assuming an unstructured covariance matrix. The primary dependent variables were six voxel-specific, coil loading-corrected, and receiver gain-corrected metabolite integrals for NAA, Cho, and Cr, and the subdomain and mean neuropsychological test performance z-scores. Data are reported as mean ± 1 SD, and the p values reported for metabolite group differences are for Cr-corrected metabolite measures derived from the BMDP analysis, unless otherwise noted. Linear relationships of metabolite values versus clinical and cognitive measures (subdomain z-scores and mean z-score across domains) were assessed using Pearson’s correlation coefficient and controlling for the effects of Cr (“partial correlation” in SAS). Results were considered significant at the 0.05 level.

Results. Subject classification. The GCIS obtained from neuropsychological tests of each individual was used for primary classification (see table 1). There were 32 HIV+ patients with a GCIS of 0 or 1 who were classified as CN, 27 HIV+ patients with a GCIS of 2 to 5 who were classified as mCI, and 11 HIV+ patients with a GCIS of 6 or greater who were classified as sCI. All 30 control subjects had GCISs of 0 (n = 18), 1 (n = 9), or 2 (n = 3), with no difference in average GCIS between high-risk and low-risk control groups or between these groups and the HIV+ CN group. Table 1 also shows the classification of HIV+ patients into CDC clinical groups: 7 belonged to CDC stage A, 36 to CDC stage B, and 27 to CDC stage C. A total of 29% of CDC A patients showed some degree of global clinical neuropsychological impairment, compared with 50% in the CDC B group and 67% in the CDC C group. The HIV+ and control groups did not differ significantly in terms of age. The HIV+ groups did not differ significantly on CD4 lymphocyte counts (see table 1).

Magnetic resonance imaging. None of the control subjects had any MRI abnormalities. Three of the 32 CN HIV+ patients (11%) and 4 of the 27 mCI HIV+ patients (15%) had mild to moderate ventricular and sulcal atrophy as well as focal white matter signal hyperintensities, primarily in the centrum semiovale. Four of the 11 sCI HIV+ patients (36%) had ventricular and sulcal atrophy and white matter abnormalities of mild to moderate degree. No significant subcortical abnormalities were noted. Paranasal sinus opacification was a common finding in many HIV+ individuals.

Metabolite measures. Thalamic and lenticular nuclei yielded spectra of good quality from more than 95% of study participants (tables 2 and 3). However, only slightly more than one-half of the subjects in this study (n = 52) had spectra from the caudate nuclei that were of sufficiently high quality to be included in the data analysis. These were distributed approximately equally across all five groups. Local magnetic susceptibility variations con-

Table 3 Neuropsychological and subcortical metabolite measures (mean of thalami, lenticular, and caudate nuclei in arbitrary units) classified by CDC clinical stage

<table>
<thead>
<tr>
<th>Group</th>
<th>GCIS, mean ± SD</th>
<th>Mean z-score ± SD</th>
<th>NAA, mean ± SD</th>
<th>Cho, mean ± SD</th>
<th>Cr, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV− pooled</td>
<td>0.5 ± 0.7</td>
<td>0.20 ± 0.38</td>
<td>45.5 ± 6.0</td>
<td>27.9 ± 4.8</td>
<td>25.3 ± 4.7</td>
</tr>
<tr>
<td>CDC A (n = 7)</td>
<td>2.1 ± 3.5</td>
<td>-0.03 ± 0.42</td>
<td>43.5 ± 6.3</td>
<td>33.3 ± 3.4</td>
<td>24.6 ± 2.0</td>
</tr>
<tr>
<td>CDC B (n = 36)</td>
<td>2.2 ± 2.5</td>
<td>-0.27 ± 0.66</td>
<td>44.3 ± 7.0</td>
<td>30.3 ± 5.9</td>
<td>24.4 ± 4.2</td>
</tr>
<tr>
<td>CDC C (n = 27)</td>
<td>2.6 ± 2.9</td>
<td>-0.41 ± 0.55</td>
<td>42.0 ± 6.6</td>
<td>31.2 ± 7.0</td>
<td>24.8 ± 5.2</td>
</tr>
</tbody>
</table>

GCIS = global clinical impairment score; NAA = N-acetylaspartate; Cho = choline-containing metabolites; Cr = creatine-containing metabolites; CDC = Centers for Disease Control.
tribute to the failure to acquire consistently good spectra from the caudate; this has been discussed in more detail earlier.  The BMDP 5V analysis treats the incomplete caudate data as though they occur at random, and allows analysis of that data along with analysis of the complete data sets from the thalamic and lenticular regions.  Although metabolite ratios were not analyzed statistically, they are listed in Table 2 to facilitate comparison with other \(^1\)H MRS studies.

Low-risk versus high-risk HIV- control subjects.  Metabolite integrals for the HIV- low-risk and high-risk control groups were not significantly different in any of the subcortical regions studied (all \(p > 0.2\)).  Therefore, the two control groups were pooled and all statistical comparisons were made relative to these pooled HIV- control subjects (\(n = 30\)).

Pooled HIV- control subjects versus pooled HIV+ patients.  The Cho integral averaged over all six subcortical locations ("subcortical Cho") was 11% higher in HIV+ patients compared with control subjects (\(p = 0.01\); see Table 2).  These results were unchanged when the caudate was excluded from statistical analyses.  The corresponding NAA and Cr integrals were not different between HIV- control subjects and HIV+ patients (both \(p > 0.2\)).  Analysis of location effects (discussed later) indicated that the observed elevation of Cho in pooled HIV+ patients relative to pooled HIV- control subjects was comparable across the thalamic, lenticular, and caudate nuclei (11 to 12%).  When correcting statistically for the variation of Cr, the group differences for subcortical Cho remained significant (\(p = 0.01\)) and subcortical NAA remained similar between the pooled HIV- and HIV+ subjects.

Location effects.  Within the subcortical structures, there were highly significant effects of location on both NAA and Cr integrals (both \(p < 0.0001\)).  For example, in pooled HIV- control subjects, thalamic NAA (51.3 \(\pm\) 7.1 in arbitrary units) was higher than lenticular NAA (49.7 \(\pm\) 7.5), which was higher than caudate NAA (34.3 \(\pm\) 3.0).  Also in control subjects lenticular Cr (27.6 \(\pm\) 6.5) was higher than thalamic Cr (25.1 \(\pm\) 4.5), which was higher than caudate Cr (21.2 \(\pm\) 6.3).  A similar pattern for both metabolites was observed for the pooled HIV+ group.  In contrast, the subcortical Cho integral, which was significantly different between pooled HIV+ patients and control subjects, showed only weak variations across the different subcortical structures within a given group (\(p = 0.03\)).  There were no location-by-group effects for any of the metabolite measures that would indicate different variation in metabolite concentration across location between the HIV- and HIV+ groups.

HIV+ groups stratified by GCIS.  Subcortical Cr was relatively unchanged across cognitive impairment-stratified HIV+ subgroups (\(p = 0.09\); see Table 2).  Subcortical Cho did not differ among CN, mCI, and sCI HIV+ subgroups (\(p = 0.90\)).  Subcortical NAA was markedly decreased with increasing severity of cognitive impairment (\(p < 0.0001\)).  The NAA integral was 20% lower in the sCI group only (\(p < 0.0001\)) and was unchanged in the CN and mCI HIV+ groups.  The Cho and NAA results were essentially unchanged when Cr values were used as a covariate to account for possible atrophy effects.

Pooled HIV- versus HIV+ subjects stratified by CDC stage.  When HIV+ patients were stratified by CDC stage (see Table 3), the GCIS and the mean impairment z-scores were not different between groups, suggesting similar levels of cognitive impairment across CDC stages in this subject cohort.  Compared with pooled HIV- control subjects, the degree of Cho elevation in the HIV+ patients was not significantly modulated by CDC staging (\(p = 0.5\)).  Similarly, subcortical NAA was not different across CDC stages (\(p = 0.2\)).  This was in contrast to classification by severity of cognitive impairment, but is consistent with the notion that cerebral metabolite measures do not reflect systemic disease primarily.

CD4 lymphocyte counts, cognitive measures, and their relationship with subcortical metabolite measures.  Although CD4 counts and subcortical Cho measures did not differ between the HIV+ groups stratified by cognitive impairment, we found moderately strong associations between low CD4 counts and high thalamic Cho in the pooled CN and mCI HIV+ cohort (\(r = -0.37, p = 0.009\)) and a weaker correlation in the entire HIV+ patient cohort (\(r = -0.27, p = 0.05\)).

The mean impairment z-score was associated with the GCIS measure of global impairment, explaining 19% of the GCIS variance when comparing HIV- control subjects with HIV+ subjects.  Impairment on psychomotor tasks explained 16% of the variance; delayed memory, 14%; abstraction and verbal, both 13%; immediate memory, 10%; motor, 9%; attention, 7%; and reaction time, 1%.  This distribution was similar when comparing mCI or sCI HIV+ groups with HIV- control subjects, with psychomotor, delayed memory, abstraction, and immediate memory explaining most of the variance, and attention and reaction time explaining the least in any of the comparisons.  Across all HIV+ patients, the mean impairment z-score and subcortical NAA were correlated, even when controlling for the effects of Cr ("partial correlations": \(r = 0.36, p = 0.003\)).  Thalamic and lenticular NAA were equally and strongly correlated with the mean impairment z-score (both, \(r = 0.32, p = 0.01\)).  Similar correlations were observed between subcortical NAA and abstraction (\(r = 0.31, p = 0.01\)), immediate memory (\(r = 0.31, p = 0.01\)), and delayed memory (\(r = 0.29, p = 0.02\)) z-scores.  Verbal (\(r = 0.29, p = 0.03\)) and attention (\(r = 0.28, p = 0.02\)) domain z-scores (the latter of which has been shown to be primarily affected early in the course of the disease and is thought to be mediated through the subcortical brain\(^1\)) were only correlated with thalamic NAA.  Performance on the Grooved Pegboard Test (testing fine motor ability and speed) showed moderately strong correlations with subcortical NAA (\(r = 0.37, p = 0.005\)), in particular with NAA from lenticular nuclei (\(r = 0.42, p = 0.001\)).  When only CN and mCI HIV+ subjects were considered in the partial correlation analyses (\(n = 59\)), the mean impairment z-score remained significantly correlated with subcortical NAA (\(r = 0.28, p = 0.03\)), and the Grooved Pegboard Test remained correlated with lenticular NAA (\(r = 0.31, p = 0.03\)).  None of the subcortical metabolite measures correlated with psychomotor impairment in the HIV+ cohort.  Subcortical Cho did not correlate with the mean z-score or any of the subdomain z-scores owing to similarly high subcortical Cho in all three HIV+ groups.

Discussion.  The major findings of this study were 1) elevated Cho and unchanged NAA in the subcorti-
cal brain of CN and clinically asymptomatic HIV+ patients compared with HIV− control subjects; 2) similarly elevated subcortical Cho in sCI HIV+ individuals, but with significantly lower subcortical NAA; 3) significant although mild correlations between subcortical NAA (but not subcortical Cho) and performance on a variety of neuropsychological tests; and 4) a lack of correlation between any subcortical metabolite measures and CDC clinical stage.

Subcortical Cho was comparably elevated throughout the basal ganglia and thalami in the entire HIV+ sample studied, and results were unchanged when the caudate was excluded from statistical analyses. High-thalamic Cho was associated with low CD4 lymphocyte counts. This elevation of Cho may reflect gliosis consistent with neuropathologic evidence of infiltration of the HIV-infected subcortical gray matter with macrophages, lymphocytes, and microglia. The lack of significant location effects on metabolite measures across the examined subcortical brain structures suggests that experimentally and analytically less demanding single-volume 1H MRS studies of thalamic or lenticular structures may be useful for HIV disease assessment.

These findings extend our earlier preliminary results to HIV+ patients without cognitive impairments or clinical symptoms. They suggest changes in subcortical cellular material early during the disease process, before neuronal damage to subcortical brain or cortical brain becomes evident. The failure to detect a greater magnitude Cho elevation in the sCI HIV+ patients may reflect either a real phenomenon or may be due to that subsample's small size and large variance. If that finding is real, it may suggest a biphasic pattern for Cho levels, with elevations resolving during the later stages of the disease (similar to recent findings for myoinositol in the frontal lobe).

Functional importance of metabolite measures. In HIV+ patients, two types of neurobehavioral disorders have been described: a dementia limited to the later stages of the disease, and a more subtle constellation of impairments that occurs in earlier stages of the disease process. In this MRS study we observed higher subcortical Cho throughout the disease process (including the early stages), and lower NAA only in later stages of the disease, when clinical impairments are evident. These contrasting findings for Cho and NAA may reflect two separable neuropathologic processes or the progression of a single process.

The mechanism and time course by which HIV produces brain functional impairments is poorly understood. Pathologic evidence suggests that HIV does not affect neurons directly, but that virus-infected macrophages damage neurons through some indirect mechanism, such as the release of toxic cytokines. Neuropathologic studies have been unable to establish an association between lower neuronal density and cognitive impairment. In this in vivo MRS study, subcortical NAA loss, which is commensurate with lower neuronal density or viability, was observed in sCI HIV+ patients, and subcortical NAA was correlated, although weakly, with measures of global clinical impairment, with specific neuropsychological test performances (abstraction, and immediate and delayed memory), and with motor function. Although present, these correlations must be interpreted cautiously because of the large number of correlations computed. However, impairment in these specific cognitive domains is consistent with another study of HIV infection performed in this laboratory that involved most of the subjects who also participated in this MRS study. We observed semantic priming deficits in cognitively impaired HIV+ patients that suggest impairment of a distributed frontocortical–subcortical network in HIV infection. Our studies are also consistent with increased latency of the orienting response occurring 300 milliseconds after an auditory stimulus (P3A) in asymptomatic HIV+ patients. Taken together, these findings paint a common picture of subcortical and frontostriatal involvement early in HIV infection when clinical and cognitive symptoms are not present.

CDC clinical stage was not associated with the severity of cognitive impairment (see table 3), suggesting a dissociation between HIV-associated cognitive impairments and HIV-associated systemic disease. This lack of an association is consistent with our finding in a previous study investigating structural changes in the brain of HIV-infected individuals.

Other functional neuroimaging modalities have also shown that the basal ganglia are frequently involved during the early stages of HIV infection (as reviewed by Navia and Gonzalez). In this study the observed Cho alterations are also consistent with subcortical neuropathology and with performance on specific neuropsychological tests, implicating early and selective involvement of subcortical and frontostriatal systems. However, we observed no association between the degree of subcortical Cho elevation and neuropsychological test performance; rather, Cho elevation preceded the development of a global clinical impairment. As such, early Cho elevation may reflect a more subtle neurobehavioral disorder in HIV-infected individuals or it may reflect the process that leads to cognitive decline during later stages of the disease.

Other MR studies of early metabolite effects in HIV infection. Most previous 1H MRS studies evaluated white matter brain regions and studied HIV+ patients primarily during relatively late stages of the disease, when severe cognitive impairments were apparent. Recently, however, with the development of more sensitive measurement techniques, more investigators have turned to study metabolite abnormalities in the brain of HIV+ patients relatively early in the disease process. Similar to the findings in this study, Tracey et al. found higher Cho ratios in the midline gray matter of the postero- parietal lobe in HIV+ patients with no or minimal cognitive impairment (absolute Cho increases were

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