Alterations in Brain Phosphate Metabolite Concentrations in Patients With Human Immunodeficiency Virus Infection

Raymond F. Deicken, MD; Bruno Hubesch, PhD; Peter C. Jensen, MD; Dominique Sappey-Marinier, PhD; Pamela Krell, PhD; Amy Wisniewski, PhD; Douglas Vanderburg, MD; Reginald Parks, MPH; George Fein, PhD; Michael W. Weiner, MD

- Human immunodeficiency virus (HIV)-infected individuals often demonstrate neuropsychiatric impairment; however, it is unclear how brain metabolism may be altered in such patients. We used in vivo phosphorus 31 magnetic resonance spectroscopy to noninvasively assess brain energy and phospholipid metabolism by measuring brain concentrations of adenine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate (Pi), as well as phospholipid compounds and intracellular pH. In study 1, 17 HIV-seropositive men with varying degrees of neuropsychiatric impairment and six control subjects were studied. Localized spectra were obtained from a heterogeneous 5 x 5 x 5-cm volume of interest (VOI). Patients with HIV infection had a significantly lower ATP/Pi and a trend for a lower PCr/Pi ratio than did the control group. In addition, the ATP/Pi and PCr/Pi ratios were both significantly negatively correlated with overall severity of neuropsychiatric impairment. In study 2, three HIV-seropositive men with neuropsychiatric impairment were compared with 11 HIV-seronegative men. Localized phosphorus 31 magnetic resonance spectra were obtained from two relatively homogeneous VOIs: (1) a predominantly white matter VOI, and (2) a predominantly subcortical gray matter VOI. The three HIV-infected patients demonstrated significantly decreased ATP and PCr concentrations in the white matter VOI. These results suggest that HIV infection of the brain may impair brain cellular oxidative metabolism and that the degree of metabolic compromise may be related to the severity of neuropsychiatric impairment.

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Compelling evidence exists that direct central nervous system (CNS) infection by the human immunodeficiency virus (HIV) occurs in a large proportion of HIV-infected individuals and often results in a neurological syndrome, acquired immunodeficiency syndrome dementia complex (ADC). This complex is characterized by disordered cognition, motor function, and behavior. About 75% of autopsies on randomly selected patients with AIDS show CNS pathology, although only about 40% of patients are neurologically symptomatic. Neurologic complications of AIDS may result from diffuse encephalopathy secondary to direct HIV infection of the CNS or from opportunistic CNS infections secondary to the immunosuppressive effects of HIV. Of 30 autopsied adult patients with AIDS, 80% had neurological signs or symptoms and 60% had histopathologic findings of subacute encephalitis; preliminary data using monoclonal antibodies against the p24 antigen in frozen brain tissue showed HIV in the CNS, especially in multinucleated cells. In over 90% of 70 autopsyed patients, abnormalities were predominantly located in the white matter and subcortical areas, with relative sparing of the cortex; progressive dementia was present in 46 patients. Human immunodeficiency virus has been detected in the white matter and basal ganglia of eight demented patients and in the cerebral spinal fluid or neural tissue of 11 of 13 patients with AIDS, where subacute encephalopathy, including monocyte/macrophage infiltration, was found. Subacute encephalitis consistent with a viral infection of the CNS was histologically defined in 12 patients with AIDS in whom the white matter was more severely affected than was the gray matter. In most cases, the HIV infection was restricted to capillary endothelial cells, mononuclear cells, and giant cells. In the brains of two patients, it was possible to identify mononucleated and multinucleated macrophages that supported replication of the AIDS retrovirus in brain tissue. In children with AIDS, study of CSF has shown HIV antigen indicating viral expression in the CNS, correlated with the occurrence of progressive encephalopathy. On the basis of culturing HIV from the cerebrospinal fluid, it was concluded that HIV is neurotropic, capable of causing acute meningitis, and responsible for...
AIDS-related chronic meningitis and dementia. Results of neuroimaging studies have consistently indicated preferential subcortical involvement in HIV infection. Subcortical structures have been shown to be affected to a greater degree than have the cortical structures on positron emission tomographic studies, at least in the initial phases of the syndrome. Magnetic resonance imaging (MRI) of patients with AIDS and patients with the AIDS-related complex (ARC) reveals multiple small and discrete lesions of high signal intensity in subcortical white matter, as well as ventricular and sulcal enlargement that is suggestive of brain atrophy.

Nava and colleagues suggested that impaired memory, concentration difficulties, and psychomotor slowing represent the most common early manifestations of ADC. This presentation is similar to that seen in other diseases with primary damage to white matter and subcortical structures. It is on this basis that ADC has been tentatively classified as a subcortical dementia. In this regard, Brouwers et al recently compared the patterns of neuropsychological performance of HIV-infected patients with those of patients with Alzheimer's disease (cortical dementia) and Huntington's disease (subcortical dementia). Discriminant analysis revealed that most patients with HIV were either classified as normal or as having Huntington's disease, while none were classified as having Alzheimer's disease. These results were not secondary to depression in a subset of patients with HIV, since the patients with HIV classified with the patients with Huntington's disease did not differ on the Beck Depression Inventory from those classified as normal. These findings are supported by the work of Van Gorp and colleagues.

In much of the emerging literature on ADC, cognitive impairment is often used synonymously with dementia, with variability across studies both on the criteria for judgment of impairment and the number of affected cognitive domains required for the diagnosis of ADC. A neurobehavioral operational definition of dementia usually includes acquired persistent impairment in at least three of the following spheres of mental activity: psychomotor control, attention and concentration, language, memory, visuospatial skills, emotion, personal- ity, and executive functions such as reasoning or cognitive flexibility. Most of the literature on ADC describes patients with varying degrees of impairment, including patients without a full-blown dementia.

Although research has been considerable on ADC in recent years, the metabolic pathology associated with HIV-related neuropsychiatric impairment is poorly understood. Rottenberg and colleagues studied regional cerebral glucose metabolism with positron emission tomography in nine patients with ADC. They found relative subcortical (thalamus and basal ganglia) hypermetabolism in early ADC, with disease progression accompanied by cortical and subcortical gray matter hypometabolism. Single-photon emission computed tomography of 12 patients with ADC revealed either multiple or focal decreases in blood flow, the latter corresponding to focal neurological signs or symptoms in all but one case. Bottomley et al reported in an abstract that in vivo phosphorus 31 magnetic resonance spectroscopy (MRS) reveals significant reductions in adenosine triphosphate (ATP) and phosphocreatine (PCr) concentrations in eight patients with ADC, compared with control subjects. Finally, Arnold et al reported that patients with a postviral exhaustion/fatigue syndrome had excessive intracellular acidosis (measured by phosphorus 31 MRS) produced by voluntary exercise of forearm flexor muscle, suggesting that viral infection, directly or indirectly produces an impairment of muscle oxidative metabolism. These studies suggest that HIV-related neuropsychiatric impairment may be associated with changes in cerebral blood flow and energy metabolism.

In an effort to further characterize the CNS metabolic alterations associated with HIV-related neuropsychiatric impairment, we utilized in vivo phosphorus 31 MRS. In vivo MRS is used to monitor intracellular metabolites noninvasively and is thought to be free of biological hazard. Moreover, because metabolites may be lost or distorted when tissue is subjected to more traditional biochemical assays such as freeze clamping, MRS may provide a more accurate assessment of concentrations of metabolites free in solution. Phosphorus 31 MRS detects the high-energy phosphates ATP and PCr and their hydrolysis product, inorganic phosphate (Pi). The ratio ATP/PCr is often calculated to indicate the "energy state" of the tissue. Phosphorus 31 MRS also detects signals from phosphodiesters (PDE) and phosphohomonoesters (PME), which provide information regarding phospholipid metabolism. Technical advances in recent years enable us to calculate absolute molar concentrations of metabolites from localized volumes of interest (VOIs) in the brain.

STUDY 1

This initial study compared a group of HIV-infected men with varying degrees of HIV-related neuropsychiatric impairment with a control group studied previously as part of an effort to determine normal brain metabolic values. All participants gave informed consent after the nature of the study had been fully explained.

Subjects and Methods

Subjects. The HIV-infected sample consisted of 17 white homosexual men, 32 to 57 years of age, from the Veterans Affairs Medical Center Infectious Diseases Clinic, San Francisco, Calif. Seven patients met the criteria for AIDS of the Centers for Disease Control, Atlanta, Ga; eight met the criteria for CDC AIDS of the Centers for Disease Control, Atlanta, Ga; and two patients were HIV seropositive, but asymptomatic. Of the patients with AIDS, four had been diagnosed with Pneumocystis carinii pneumonia, two with lymphoma, and one with Kaposi's sarcoma. Patients were excluded from the study if they had a history of recent head injury, seizure disorder, multiple sclerosis, cerebrovascular disease, or organic brain disorder. Two patients were being treated with zidovudine (azidothymidine) alone and 10 patients were being treated with zidovudine plus other antiviral agents such as acyclovir.

The control group consisted of five men and one woman, 29 to 44 years of age, all without a history of recent medical illness, neurological disease, or psychiatric disorder, or substance abuse. No members of the control group belonged to any group at high risk for HIV infection. These subjects had been studied 6 months previously as control subjects in a study of brain tumors.

Assessment of ADC. All HIV-infected subjects underwent neuropsychological, psychiatric, and neurological examinations. Neuropsychological testing included measures of orientation, attention, language, spatial construction skills, calculation, reasoning, and memory. Visual motor coordination, motor speed, and grip strength were assessed to appraise perceptual-motor function. All measures were scored using standardized procedures incorporating age and education corrections when such were available for measure. Psychiatric assessment consisted of a structured psychiatric interview, the Brief Psychiatric Rating Scale, Beck Depression Inventory, and the Symptom Checklist-90. The neurological status of each patient was assessed utilizing the AIDS Treatment Evaluation Unit (ATEU) Neuro-AIDS Assessment Form, which provides a more quantitative than a descriptive method of evaluating the neuropsychological and neurobehavioral consequences of HIV infection. The deficit score was derived from the sum of the impairment on each test for a given neuropsychological or neurobehavioral domain.

MRS and clinical assessments were performed on a General Electric Signa 1.5-T MRI scanner at the Human Brain Laboratory. Absolute molar concentrations of metabolites are calculated using a two-point method. The metabolite concentrations are calculated using the ratio of the areas of the observed resonances and the known resonance characteristics of each metabolite. The resonances are referenced to that of phosphodiesters (PDE), phosphomonoesters (PME), and inorganic phosphate (Pi).

Results of neuroimaging studies...
quantitative measure of neurological status than can be obtained from a standard descriptive neurological examination. Utilizing the information obtained from the neuropsychological, psychiatric, and neurological evaluations, each subject was given a summary assessment score for cognitive impairment, behavioral disturbance, and neurological impairment on a four-point scale (0, none; 1, mild; 2, moderate; and 3, severe) utilizing specific parameters established in the ATEU Neuro-AIDS Assessment Form. An overall composite dementia score was then computed for each patient by averaging the cognitive, behavioral, and neurological function summary assessment scores.

MRI and MRS. — All studies were performed in the San Francisco Veterans Affairs Medical Center using a Philips Gyroscan 2.0-T S15 MRI/MRS system. A standard imaging saddle-type proton head coil was used for MRI. For phosphorus 31 MRS, a 16-cm-diameter closely coupled head coil composed of two circular loops was used. Subjects were positioned supine in the magnet with the head inside both coils; the radiofrequency fields of the two coils were orthogonal to each other so that the coils did not interact. A 3-ml external reference of hexamethylphosphor只是amide (HMPT) was positioned in the center of one of the loops of the phosphorus 31 coil. This reference was used (1) to confirm coil position on the magnetic resonance image; (2) to determine the 90° pulse length at the reference position (so that the 90° pulse length at the center of the VOI could be set using a computer-generated B-1 field plot); and (3) as a reference for the relative sensitivity of the loaded coil for the calculation of metabolite concentrations. To accurately select the volume of interest displayed in Fig 1. 5 x 5 x 5-cm volume of interest was chosen to coincide with the VOI position was determined from the phosphorus 31 signal (which yielded the control data) and is displayed in a one-pulse spectrum of the HMPT standard. The magnet was shimmed by observing the proton signal of water (85.9 Hz at 2.0 T) using a computer-generated B-1 field plot. A one-pulse spectrum of the HMPT standard was recorded with a 90° excitation pulse for calibration of the loaded coil sensitivity. Finally, phosphorus 31 spectra were acquired using the improved image-selected in vivo spectroscopy (ISIS) method at 2.0 T. Acquisition parameters were as follows: repetition time, 2 seconds; sweep width, 2 kHz; number of scans, 640; and data size, 1000.

Phosphorus 31 data were processed using the NMR-1 program on a Sun Systems workstation. The broad signal from the less-mobile metabolites was removed by convolution difference and baseline flattening procedures. In addition, an exponential line-broadening factor of 5 Hz was used. Integrals from individual resonance signals were then obtained from least-squares curve-fitted spectra and used for calculation of concentrations and ratios of peak integrals with correction for T saturation. The correction for T saturation is especially important for PCR, for which the pulse repetition time (TR) is less than 2 T. For calculation of brain metabolite concentrations in millimoles per liter, an 80% water content was assumed. The metabolites assessed included ATP, Pi, PCR, PDE, and PME. A sample spectrum from a patient with HIV is shown in Fig 2.

Results. — The pH was calculated from the chemical shift of inorganic phosphate (δPi) referenced to the chemical shift of phosphocreatine (δPCR), which was assumed to be 0 ppm, where δPi and δPCR were obtained from the curve-fitted peak positions:

\[ pH = 6.77 + \log(\delta Pi - 3.29)/(5.68 - \delta Pi) \]

Results. — Data were initially analyzed to determine if the 17 HIV-infected patients had different concentrations of...
high-energy phosphates compared with the normal control subjects. The ATP/Pi ratio in brain was lower for the HIV patients compared with the normal control subjects (1.45 ± 0.45 vs 2.07 ± 0.50, P = .03). The absolute magnitude of the difference between groups was the same for the PCr/Pi ratio, but the variance of the ratios was somewhat larger (2.32 ± 0.77 vs 2.96 ± 0.83, P = .10). These comparisons were unchanged when examined using nonparametric statistics. These differences between the samples were due to lower ATP and PCr concentrations and slightly higher Pi concentrations across all brain VOI. There were no significant differences between patients with HIV and control subjects concerning PME or PDE concentrations or the ratios involving these measures.

**MRS and Composite Dementia Scores.**—When the high-energy phosphates of both the controls and patients with HIV were examined in relation to the normal control summary assessment scores, a number of significant correlations resulted. The ATP/Pi ratio had a significant negative correlation with each of the summary assessment scores (r = −0.63, P = .007; r = −0.44, P = .04; and r = −0.46, P = .03; and r = −0.07, P = .005 for the cognitive, neurological, behavioral, and composite dementia scores, respectively). A scatterplot of the ATP/Pi ratios vs the cognitive impairment summary score is displayed in Fig 3. Comparable significant negative correlations were noted between the PCr/Pi ratios and the summary assessment scores (r = −0.65, P = .007; r = −0.53, P = .01; and r = −0.47, P = .03 for the cognitive, neurological, behavioral, and composite dementia scores, respectively). There were no significant correlations between any of the phospholipid metabolite measures and any of the composite dementia scores.

**MRS and Illness Severity.**—There were no significant group differences in ATP/Pi or PCr/Pi ratios for patients with AIDS vs patients with ARC. In addition, of nine patients who had CD4 lymphocyte measurements obtained at the time of the study, there were no significant correlations between ATP/Pi or PCr/Pi ratios and absolute CD4 lymphocyte counts. Weight loss was also examined in relation to the ATP/Pi ratio. Three patients had a weight loss of 2.25 kg (5 lb) or greater in the 6 months before enrolling in the study. The ATP/Pi ratio for these patients was 1.51 ± 0.62, which was not significantly different from the corresponding values for the entire ARC group (1.45 ± 0.45).

**Cerebral Atrophy.**—Eleven of 17 HIV-infected patients had full T2-weighted MRS studies that were evaluated for evidence of cerebral atrophy and other CNS pathology. Five patients had evidence of atrophy, and no other abnormalities were noted. Measurements of cerebral atrophy were not significantly correlated with the ATP/Pi and PCr/Pi ratios or ATP or PCr concentrations. However, ventricular enlargement was positively correlated with pH (r = .78, P = .007) and negatively correlated with the PME concentration (r = −.63, P = .088). Sural enlargement scores were positively correlated with the cognitive disturbance summary scores (r = .65, P = .08).

**COMMENT**

Although preliminary, the results of study 1 suggest a decrease in brain oxidative metabolism in patients with HIV infection, with the magnitude of the effect directly associated with the magnitude of the patient's neuropsychiatric impairment. We do not believe that the MRS changes are the result of more cerebrospinal fluid in the brain VOI of patients with neuropsychiatric impairment because (1) measurements of cerebral atrophy were not significantly correlated with the ATP/Pi or PCr/Pi ratios or ATP concentration; (2) MRS studies of dementias in the elderly do not report decreased ATP/Pi ratios in the presence of cortical atrophy; (3) our examination of two sagittal images through the lateral ventricles in 10 patients in study 1 (the five with the highest and lowest ATP/Pi ratios did not reveal any correlation between the total ventricular and sulcal areas within the MRS VOI and the ATP/Pi ratios); and (4) the concentration of Pi in cerebrospinal fluid (0.17 mol/L) is an order of magnitude smaller than the concentration in brain, and the concentration of ATP in cerebrospinal fluid is even smaller; thus, the effect of relatively small differences in the proportion of cerebrospinal fluid in the MRS volume should be to decrease the absolute concentrations, with little, if any, effect on ratios. Finally, although the quantitation of metabolite concentrations requires assumptions regarding the tissue water content, and it is possible that tissue water content differs between groups, such differences would have to affect concentrations of all metabolites equally and could not underlie the specific changes we observed in ATP and PCr concentrations.

Because the 5 × 5 × 5-cm VOI is a heterogeneous volume containing substantial contributions from white matter, gray matter, and ventricular cerebrospinal fluid, and because the weight of histological and imaging studies indicates preferential subcortical involvement by HIV in the CNS, we refined our MRS procedures for study 2 to determine whether the MRS changes are specific to white matter or subcortical gray matter. In addition, preliminary data from our laboratory suggest that phosphorus metabolite concentrations are strongly affected by varying the brain region studied. Thus, we believed that studying more homogeneous VOIs would yield less variation in metabolite concentrations across subjects within each group, increasing the statistical power of group comparisons. For study 2, we also selected a more appropriate control group, age and sex matched to the patients with HIV, but with documented HIV seronegativity.

**STUDY 2 Subjects and Methods**

**Subjects.**—In the second part of our ongoing study, we have, so far, studied 12 heterosexual male control subjects (mean age ± SD, 24.8 ± 4.4 years) in whom HIV seronegativity was confirmed by HIV-anti-body testing (enzyme-linked immunosorbent assay) and three HIV-infected patients with moderate-to-severe neuropsychiatric impairments. All control subjects were examined and found to be cognitively unimpaired using the same neuropsychological battery as used for the examinations of the HIV-infected patients. Inclusion and exclusion criteria were as described for study 1 subjects. All three HIV-infected patients carried a diagnosis of ARC, and two patients were receiving zidovudine at the time of study.

**MRS and MRS.**—Procedures were identical to those described in study 1 except that we selected two different VOIs to better characterize metabolic alterations in the subcortical gray matter and white matter. The subcortical gray matter

**For the white matter infected patients, both the ATP and PCr concentrations were studied for both. Mann-Whitney U tests for differences in the study 2 subjects.**

**CONCLUSIONS**

Neuropsychiatric impairment in HIV infection is associated with decreased brain oxidative metabolism, decreased ATP levels, and increased Pi levels. These observations are consistent with the hypothesis that the primary pathogenesis in HIV infection is an imbalance between oxidative metabolism and glycolysis. Because the MRS changes were more pronounced in subcortical gray matter, the metabolic changes may underlie the primary cognitive impairment in HIV infection.

**Fig. 5.**—Phosphorus magnetic resonance spectroscopy (MRS) 

**Fig. 4.**—These two subcortical gray matter VOIs.
Results

For the white matter VOI, the HIV-infected patients evidenced decreased ATP and PCr concentrations (P = .036 for both, Mann-Whitney U test) and trends for decreased ATP/Pi ratios and PCr/Pi ratios (P = .073 for both, Mann-Whitney U test). Metabolite concentrations and ratios are displayed in Fig 5. For the subcortical gray matter VOI, no differences between the ADC and control groups were apparent on any variables. The SEs of the absolute metabolite concentrations were smaller (about 5% to 7% of the mean value) than were the SEs for the white matter VOI and two periventricular subcortical gray matter VOIs. Although we studied only three patients for the white matter VOI and two patients for the subcortical gray matter VOI, the decrease in ATP and PCr concentrations appeared to be specific to the white matter VOIs. Although this very preliminary result clearly requires confirmation with a larger sample size, it is in agreement with neurohistological and MRI data revealing major HIV-related neuropathology in white matter.13,16

The results from study 1 also suggest that the degree of metabolic compromise may be related to the severity of neuropsychiatric impairment, as evidenced by significant negative correlations between the ATP/Pi and PCr/Pi ratios and the neuropsychiatric summary assessment scores. This is the first in vivo MRS study, to our knowledge, to report an association between the severity of HIV-related neuropsychiatric impairment and the degree of impaired brain energy metabolism. This finding is consistent with the results of Pohl et al,29 who noted that worsening of dementia in patients with ADC was associated with an increase in uptake defects (decreased local cerebral blood flow and disturbed cerebral amine metabolism) on single-photon emission computed tomography. If this association is corroborated by further study, in vivo phosphorus 31 MRS may hold promise for monitoring biological factors associated with HIV disease progression in the brain and for monitoring response to treatment with medications that improve HIV-related neuropsychiatric impairment, such as zidovudine.23,24

The association of HIV-related neuropsychiatric impairment with changes in brain high-energy phosphorus metabolism suggests two nonexclusive possibilities. The first is that the metabolic alterations are due to a severe metabolic disturbance in focal areas with the VOI. The second possibility is that the metabolic alterations are due to a process that affects most or all cells within the VOI. To determine if the nature and extent of HIV-related MRS-determined metabolic

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terations are due to focal lesions, we are currently attempting to correlate MRI findings (brain atrophy and white-matter disease) with MRS results. If we find that MRS detected changes that occurred in VOIs with absent or very few focal MRI lesions, this will be interpreted to suggest that the metabolic changes are due to a process that affects most cells within the VOI.

It is unclear at this time whether altered brain phosphorus metabolism is specific to HIV disease or is a general finding reflective of a progressive subcortical dementia process. There have been very few in vivo phosphorus 31 MRS studies of subcortical dementia syndromes such as Parkinson's or Huntington's dementia. One small study of Parkinson's disease found no significant differences in the ratios of the various phosphorus metabolites such as PCr/Pi compared with control subjects. Nevertheless, it is conceivable that our findings of impaired energy metabolism could be the consequence of the subcortical nature of HIV-related neuropathy and would be present in other subcortical dementia syndromes as well. Therefore, we are currently undertaking in vivo phosphorus 31 MRS studies of non-HIV-related subcortical dementias (such as multiple sclerosis-related dementia) to address this issue. In vitro and in vivo phosphorus 31 MRS studies of cortical dementias such as Alzheimer's and multi-infarct dementia have revealed alterations primarily in the PDE and PDE resonances with no significant changes in measurements or indexes of cellular oxidative metabolism.28,29,30

Our ongoing in vivo phosphorus 31 MRS study of deep white-matter lesions (also called leuko-araiosis) in demented and nondemented elderly subjects also provides interesting comparative data. In white matter VOIs, we have found significant reductions in the ATP/Pi ratios and PDE concentration associated with the presence of extensive deep white-matter lesions, but unrelated to the presence of dementia.30 In the current study, where many of the HIV-infected patients also have white-matter lesions evidenced on MRI, we find a similar decrease in the ATP/Pi ratio but fail to find a decrease in the PDE concentration. This may indicate that the mechanisms underlying the white-matter changes differ in the two disease processes or may reflect a difference in the severity of disease in the two cases. This study illustrates the importance of quantitation methodology, along with image-guided three-dimensional localized phosphorus 31 MRS. We used an improved ISIS technique to obtain MRI-localized phosphorus 31 MRS of human brain. This technique, which has been carefully studied in computer-simulated analyses and phantom studies,31 provides accurate localization for characterization of relatively homogeneous human brain VOIs.

There are a number of simplifying assumptions that, as we have noted previously,32 are involved in the calculation of molar concentrations. First, for quantitation, the tissue within the VOI is assumed to be homogeneous, with the measured concentrations representing the mean concentration within the VOI. Second, the T1 values used to calculate concentrations were derived from normal subjects for large brain volumes, which were less homogeneous than the VOIs of the current study. This was necessary because of the tremendously lengthy study times necessary to measure phosphorus 31 T1 values. We estimated that a T1 study for each metabolite separately for the white matter and subcortical gray matter VOIs would involve having each subject spend several hours in the magnet for each VOI. Third, our measurements could be influenced by variation between subjects in metabolite mobility as well as in metabolite concentration. Fourth, we assumed a uniform tissue water content for each VOI across subjects. If the water content varied among subjects, that could have affected the concentrations, but it would have affected all metabolites equally.

The simplifying assumptions noted above argue for caution in the interpretation of our results. The results clearly indicate that there are changes in aspects of the phosphorus 31 MRS associated with HIV infection. Although it is possible that the effect reflects differential changes in the mobility of the various metabolites, or in their T1 values, we do not believe it is likely. Our preliminary simulations suggest that the effect of differences between metabolites in mobility and T1 values would be small compared with the effect of differences in metabolite concentration (except possibly for PCr, where the T1 time is greater than the repetition time). Thus, the most parsimonious interpretation of our results is that they reflect concentration differences.

Finally, it is important to note that in vivo phosphorus 31 MRS technique is in its infancy. We fully expect great advances in signal strength, pulsing technology, and phosphorus 31 spectroscopic imaging. These advances will result in the ability to quantitate metabolite concentrations throughout the brain in ever smaller voxels. Such advances, together with the development of proton MRS technology, portend an explosion of in vivo studies of brain metabolism in normal and pathological conditions.

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