Journal of NeuroVirology, 11: 356–364, 2005

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280591002342

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Brain mitochondrial injury in human immunodeficiency virus–seropositive (HIV+) individuals taking nucleoside reverse transcriptase inhibitors

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Nucleoside reverse transcriptase inhibitors (NRTIs) suppress human immunodeficiency virus (HIV) replication, but are often associated with mitochondrial toxicity. Although well studied outside of the central nervous system, no investigation has examined the effects of these drugs on brain mitochondria of individuals living with HIV. The authors used proton magnetic resonance spectroscopy to evaluate NRTI-related changes in brain mitochondria. *N*-acetylaspartate (NAA; sensitive to alterations in mitochondrial integrity) was measured in frontal lobe white and gray matter of 18 HIV+ individuals taking didanosine and/or stavudine (two NRTIs likely to cause mitochondrial toxicity), 14 HIV+ individuals taking zidovudine and lamivudine, 16 HIV+ individuals not currently taking antiretrovirals, and 17 HIV− controls. The HIV+ groups were comparable on demographic measures, estimates of illness severity, and estimated length of HIV infection. Those taking didanosine and/or stavudine had a significant 11.4% decrease in concentrations of frontal white matter NAA compared to HIV− controls, whereas NAA levels of the other HIV+ groups were intermediate. Group differences in metabolites were not found in frontal gray matter. Lower levels of frontal white matter NAA were associated with longer periods of didanosine and/or stavudine treatment ($r = -.41$, $P = .06$). Levels of NAA were not related to length of zidovudine/lamivudine treatment ($r = -.04$, $P = .44$). Furthermore, taking more than one of stavudine, didanosine, and abacavir increased the likelihood of having reduced NAA. The results are consistent with previous studies finding HIV-related changes in neuronal integrity. However, because NRTIs can injure mitochondria, we propose that the observed reductions in NAA in individuals taking didanosine and/or stavudine may be the result of depleted brain mitochondria and/or alterations in cellular respiration. Measurement of brain metabolites sensitive to impairments in energy metabolism, including NAA, may aid in early detection of subclinical NRTI-mediated mitochondrial toxicity. *Journal of NeuroVirology* (2005) **11,** 356–364.

Keywords: brain magnetic resonance spectroscopy (MRS); human immunodeficiency virus (HIV); mitochondrial toxicity; *N*-acetylaspartate; nucleoside reverse transcriptase inhibitor (NRTI)

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This work was supported by DA12065 from the National Institute of Drug Abuse and MH62512 from the National Institute of Mental Health to Dr. Igor Grant.

The authors would like to thank Dexter Walpole and Jennifer Marquie-Beck for their valuable assistance in data collection and preparation.

Received 23 March 2003; revised 7 March 2005; accepted 6 April 2005.

The nucleoside reverse transcriptase inhibitors (NRTIs) are derivatives of adenosine, cytidine, guanosine, and thymidine and are phosphorylated within cells to dideoxynucleoside triphosphates. Phosphorylated NRTIs (NRTI-TP) act as competitive substrates for human immunodeficiency virus (HIV) reverse transcriptase. Their incorporation into a growing DNA strand results in premature DNA chain termination, preventing viral reverse transcriptase transcription and formation of provirus. Although this provides an effective mechanism for inhibition of virus replication, the NRTI-TP can also be incorporated into human polymerases used for DNA transcription and replication (Furman *et al*, 1986; Lim and Copeland, 2001).

Although all DNA polymerases can incorporate NRTI-TP into the chain, polymerase gamma seems particularly susceptible, possibly because of RNA-dependent activity similar to HIV reverse transcriptase (Naviaux *et al*, 1999). Polymerase gamma is found within mitochondria and is solely responsible for mitochondrial replication. This enzyme incorporates zalcitabine, didanosine, and stavudine more readily than other NRTIs such as lamivudine, zidovudine, and abacavir (Martin *et al*, 1994). These three drugs are, therefore, more likely to interfere with mitochondrial DNA synthesis, leading to adverse reactions. Depletion of mitochondria through this mechanism is thought to be at least partially responsible for many side effects associated with nucleoside analog treatment, including peripheral neuropathy, hyperlactatemia, and lactic acidosis. However, the impact of NRTIs on central nervous system (CNS) integrity, particularly in individuals living with HIV, is generally unknown. There is a clear need to be able to monitor these changes *in vivo*, as changes in CNS mitochondria may contribute to the neurologic and cognitive deficits often seen in HIV+ individuals.

Proton (^{1}H) magnetic resonance spectroscopy (MRS) has consistently demonstrated an HIV-associated reduction in *N*-acetylaspartate (NAA). Immunohistochemical studies have localized NAA to neurons (Moffett *et al*, 1991; Simmons *et al*, 1991), and it has been reported to be an axon-specific marker (Bjartmar *et al*, 2002). Thus, it appears to be a reliable *in vivo* marker of neuronal integrity. Additionally, NAA may also serve as a marker of *mitochondrial integrity*. NAA reductions have been observed during adenosine triphosphate (ATP) inhibition and impaired oxygen consumption in isolated mitochondria *in vitro* (Bates *et al*, 1996). In addition, Demougeot and colleagues (2001) reported that 3-nitropropionic acid (3NP), which inhibits the succinate dehydrogenase step of the tricarboxylic acid cycle, reduced concentrations of rat striatal NAA in the absence of neuronal loss. These results suggest that NAA loss may be more directly attributable to mitochondrial damage than to actual neuronal loss. Because NAA synthesis and ATP production are strongly linked (Bates *et al*, 1996), even subtle changes in brain energy metabolism may reduce NAA.

Although some investigators have used MRS to monitor response to antiretroviral treatment, no study has specifically addressed the impact of NRTI-related mitochondrial toxicity on brain metabolism. Because didanosine and stavudine potently inhibit mitochondrial DNA polymerase gamma, we hypothesized that HIV+ individuals taking an antiretroviral regimen that included these NRTIs would have reduced concentration of NAA in frontal lobe white matter and gray matter, compared to HIV+ individuals taking zidovudine + lamivudine as part of a treatment regimen, HIV+ individuals not taking antiretrovirals, and HIV− control participants. Based on our hypothesis, we also expected that longer durations of didanosine and/or stavudine therapy would correlate with the degree of reduction in NAA.

Results

Table 1 displays means and standard deviations for metabolites in frontal white and gray matter. In the frontal lobe white matter (FWM) region of interest, analysis of variance (ANOVA) revealed significant group mean differences for concentrations of NAA ($F(3, 64) = 3.04$, $P = .04$, $\eta^2 = .13$). Pairwise comparisons showed HIV+/d* (see Materials and Methods for definition) had significantly lower NAA compared to HIV− individuals ($P = .03$). HIV+/naïve displayed a trend toward lower NAA, relative to HIV− controls ($P = .14$). HIV+/ZDV had concentrations of NAA intermediate to HIV− controls and HIV+/d*.

Significant group mean differences were also found for concentrations of FWM creatine + phosphocreatine ($F(3, 64) = 2.76$, $P = .05$, $\eta^2 = .12$). HIV+/d* had lower creatine + phosphocreatine compared to HIV− individuals ($P = .06$). However, there appeared to be a general trend towards lowered concentrations of creatine + phosphocreatine in all HIV+ groups. No group mean differences were found for concentrations of choline-containing compounds ($F(3, 64) = 1.74$, $P = .17$) or *myo*-inositol ($F(3, 62) = 0.24$, $P = .87$).

The HIV+ groups and HIV− controls had comparable concentrations of metabolites in the frontal lobe gray matter region of interest. No significant group mean differences were found for NAA ($F(3, 64) = 0.74$, $P = .53$), creatine + phosphocreatine ($F(3, 64) = 0.11$, $P = .95$), choline-containing compounds ($F(3, 64) = 0.20$, $P = .90$), or *myo*-inositol ($F(3, 64) = 1.68$, $P = .18$).

Correlation analysis (one-tailed) revealed that subjects with a longer duration of stavudine and/or didanosine treatment prior to the spectroscopy examination had lower concentrations of NAA in frontal white matter ($n = 15$, $r = -.41$, $P = .06$). Duration of zidovudine therapy prior to the examination was not

Table 1 Group means and standard deviations for *N*-acetylasparate (NAA), choline-containing compounds (Cho), creatine + phosphocreatine (Cr), and *myo*-inositol (Ins) in frontal white matter (FWM) and frontal gray matter (FGM)

	FWM				*FGM*			
	*HIV+/d**	*HIV+/ZDV*	*HIV+/naïve*	*HIV−*	*HIV+/d**	*HIV+/ZDV*	*HIV+/naïve*	*HIV−*
NAA	7.31	7.76	7.51	8.25	7.01	7.38	7.21	7.30
	(1.12)	(0.86)	(0.96)	(0.79)	(0.87)	(0.70)	(0.75)	(0.67)
Cho	1.54	1.68	1.68	1.77	1.28	1.34	1.32	1.33
	(0.31)	(0.27)	(0.27)	(0.36)	(0.26)	(0.32)	(0.24)	(0.24)
Cr	4.74	4.78	4.84	5.36	4.97	5.03	5.02	5.12
	(0.56)	(0.52)	(0.63)	(1.00)	(0.73)	(0.81)	(0.71)	(0.80)
Ins	4.26	4.08	4.07[a]	4.39[a]	3.79	3.93	3.74	4.26
	(0.83)	(1.21)	(1.02)	(1.69)	(0.68)	(1.02)	(0.65)	(0.64)

[a]Analyses for Ins in FWM had sample sizes of 15 for HIV+/naïve and 16 for HIV−.

associated with frontal white matter NAA ($n = 13$, $r = -.04$, $P = .44$) (Figure 1). Four individuals were excluded from this analysis because they were unable to provide the particular month they began using NRTIs.

We completed post hoc analyses within the HIV+/d* group to examine the impact of NRTI load on frontal white matter NAA. Individuals who took only one of the following: (1) d4T or (2) ddI were placed in one group, HIV+/d1. Individuals taking two or more of the following: (1) d4T, (2) ddI, or (3) abacavir where placed in a second group, HIV+/d2. We did not base groupings on the presence of zidovudine or 3TC, as we hypothesized that these drugs would have minimal effect on neuronal mitochondrial functioning. *t* test revealed that HIV+/d2 had a trend toward lower NAA in the frontal white matter region of interest, relative to HIV+/d1 (HIV+/d1, $n = 8$, mean NAA = 7.87, SD = 1.11; HIV+/d2, $n = 10$, mean NAA = 6.85, SD = 1.09; $t(16) = 1.95$, $P = .07$). HIV+/d1 and HIV+/d2 had similar CD4 counts (HIV+/d1 CD4 mean = 433.1, SD = 292.2 and HIV+/d2 CD4 mean = 468.4, SD = 206.3; $t(16) = -0.29$, $P = .78$). There was a trend toward increased plasma HIV viral load in the HIV+/d2 group ($P = .10$). However, estimates of cerebrospinal fluid viral load, which may be a better measure of virus copies in the central nervous system, were nearly identical between groups ($P = .96$).

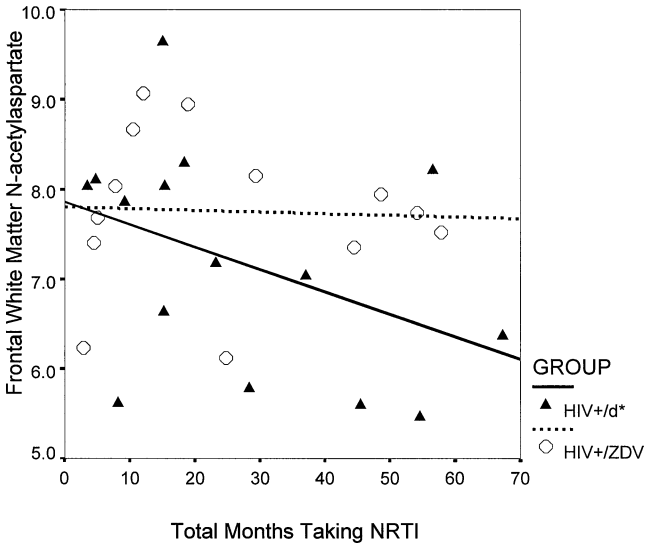

Figure 1 The scatterplot displays total months taking nucleoside reverse transcriptase inhibitors prior to spectroscopy examination, predicting concentrations of frontal white matter *N*-acetylaspartate. Individual regression lines for HIV+ individuals taking stavudine and/or didanosine ($r = -.41$, $P = .06$) and HIV+ individuals taking zidovudine + lamivudine are shown ($r = -.04$, $P = .44$).

Further analyses were completed to investigate the relationship between concentrations of frontal white matter NAA and age, immunosuppression, and viral load for participants. Significant relationships or trends were not found between NAA and age for each group. In addition, HIV+ individuals did not show significant correlations between NAA and CD4 counts or viral burden in plasma or CSF.

Discussion

HIV+ individuals taking an antiretroviral regimen that included stavudine and/or didanosine (HIV+/d*) had a significant 11.4% decrease in frontal white matter NAA compared to HIV− controls. Although not statistically significant, HIV+/d* had a 5.8% reduction in frontal white matter NAA when compared to HIV+ individuals taking zidovudine and lamivudine and a 2.7% decrease compared to those not taking antiretrovirals at the time of the scan. Furthermore, HIV+/naïve had a trend toward lower NAA in frontal white matter compared to HIV− controls, and, in general, lower NAA compared to HIV+ individuals taking zidovudine and lamivudine in the same region of interest. This was despite the fact that HIV+ groups were comparable on demographic measures, estimates of illness severity, and estimated length of HIV infection. There was a significant relationship between length of NRTI treatment and frontal white matter NAA in HIV+/d*. Longer

periods of treatment were associated with greater reductions in NAA. Furthermore, individuals taking more than one of the following NRTIs (stavudine, didanosine, or abacavir) showed a trend toward lower NAA compared to HIV+ individuals taking either didanosine or stavudine in the context of an antiretroviral regimen, suggesting a potential NRTI load on neuronal change. Decreased levels of NAA in individuals taking stavudine and/or didanosine may represent NRTI-related mitochondrial toxicity in the brain.

Infection with HIV has been associated with reductions in NAA. This change has been found in HIV+ individuals with acquired immunodeficiency syndrome (AIDS) dementia complex (Menon *et al*, 1992; Barker *et al*, 1995; Laubenberger *et al*, 1996; Tracey *et al*, 1996; Lopez-Villegas *et al*, 1997; Salvan *et al*, 1997a, 1997b; Möller *et al*, 1999), cognitive impairment (Meyerhoff *et al*, 1993, 1994), leucoencephalopathy/encephalitis (Wilkinson *et al*, 1996), neurological symptoms (Paley *et al*, 1995, 1996), focal brain lesions (Chang *et al*, 1995; Simone *et al*, 1998; Iranzo *et al*, 1999), and medically asymptomatic HIV+ individuals (Laubenberger *et al*, 1996; Wilkinson *et al*, 1997). Collectively, these studies indicate that levels of NAA are reduced in both neurologically asymptomatic and impaired HIV+ patients, and this change is thought to be related to neuronal loss or injury. The results from this study are consistent with these findings.

Antiretroviral treatment with zidovudine can increase amounts of NAA. In one investigation, initial reductions in NAA/creatine were reversed by zidovudine treatment in three patients with AIDS dementia complex (Vion-Dury *et al*, 1995). In contrast, Chang and colleagues (1999) revealed that whereas highly active antiretroviral treatment for a 3- to 14-month duration resulted in changes in levels of choline and *myo*-inositol that approximated control values; NAA levels did not significantly change during treatment. Interestingly, 8 of the 16 individuals in this study were placed on an antiretroviral regimen that included didanosine and/or stavudine. It seems possible that the lack of NAA change could be related to the mitochondrial toxicity of these drugs.

Kakuda (2000) suggests there may be multiple pathways of nucleoside-induced mitochondrial toxicity. These include mitochondrial DNA chain termination, competitive inhibition, impairments in proteins encoded by the mitochondrial genome including electron transport subunits, changes in enzymatic activities of adenylate kinase and adenosine diphosphate/adenosine triphosphate translocase, alterations in oxidative phosphorylation, and apoptosis.

Two particularly interesting mechanisms of NRTI-mediated changes of NAA may be competitive inhibition of mitochondrial replication and disruption of mitochondrial electron transport. Mitochondria may be depleted when polymerase gamma is inhibited within cells. NAA is produced in mitochondria from aspartate and acetyl–coenzyme-A, catalyzed by L-aspartate-*N*-acetyltransferase in an energy dependent reaction. Reduction in number of neuronal mitochondria, as seen in NRTI-induced peripheral neuropathy, would likely result in NAA changes in the central nervous system. As there is little evidence to suggest a mechanism of NAA synthesis up-regulation during periods of decreased concentration, reductions in mitochondrial number associated with continuous NRTI treatment may result in a direct and prolonged decrease in NAA concentration. Haas (2000) estimated that a 70% reduction in mitochondrial DNA may be needed to exert clinical effects, but MRS may detect subclinical changes in mitochondria and NAA. MRS measurement of brain NAA, as well as other markers of energy states, including lactate and ^{31}P MRS-detected levels of nucleoside triphosphate and phosphocreatine, along with clinical indicators of peripheral and central nervous involvement may uncover NRTI-related injury earlier than traditional clinical markers alone.

A second mechanism that may underlie the observed change in NAA may be NRTI-induced changes in electron transport within mitochondria. The electron transport chain (ETC) is involved in a series of redox reactions which provide energy for ATP synthesis. Decreased NAA synthesis has been shown in the presence of ETC inhibitors. Inhibition of complexes I, III, IV, and V of the ETC has been shown to diminish oxygen consumption and ATP synthesis in isolated brain mitochondria, resulting in substantially decreased NAA (Bates *et al*, 1996). Experimental rodent models of brain injury show similar relationships between production of ATP and NAA, further supporting the relationship between NAA and energetic states (Signoretti *et al*, 2001). Didanosine has been shown to interfere with complex III in hepatocytes (Youssef and Badr, 1992; Pan-Zhou *et al*, 2000), and zalcitabine, didanosine, and zidovudine altered complex II and IV activities in human muscle cells (Benbrik *et al*, 1997). Inhibition of mitochondrial electron transport complexes in neurons may be an alternative mechanism of NRTI-mediated decreases in NAA.

In summary, the lower NAA concentrations observed in individuals taking didanosine and/or stavudine are consistent with the notion that these drugs injure mitochondria. The results are also consistent with the results of previous MR spectroscopic studies showing HIV-related changes in neuronal integrity. Our findings support a length of treatment-response relationship because NAA levels were lowest in those with the longest didanosine and stavudine exposures. Furthermore, taking more than one of stavudine, didanosine, or abacavir increased the likelihood of having reduced NAA. Differences in disease severity are not thought to explain the differences, because the HIV+ subjects had comparable levels of CD4+ lymphocytes and hemoglobin. The

antiretroviral-treated groups also had similar HIV RNA levels.

It is unclear whether differences in the blood-brain barrier (BBB) penetration of NRTIs accounts for changes in concentrations of NAA. Didanosine, stavudine, zalcitabine, and zidovudine displayed similar permeability coefficients crossing model BBB microvessel endothelial cells (Glynn and Yazdanian, 1998). However, these models may not accurately represent the *in vivo* situation.

Several limitations must be considered when interpreting the results. The study was retrospective in nature and had a relatively small sample size. A prospective analysis during which HIV+ individuals are placed on and off these antiretrovirals at variable doses would clarify the results of this study and determine the reversibility of changes in NAA. A study of this design would permit evaluation of the relationship between changes in brain metabolism and cognitive abilities. In addition, adherence was not strictly monitored in this study, but we do not expect between group differences in medication adherence. Individual differences in brain antiretroviral metabolism should be considered as some HIV+ patients could experience greater toxic effects. For example, some patients experience peripheral neuropathy as result of treatment whereas others do not. The results of this study are preliminary and suggestive but not conclusive.

Materials and methods

Subjects

Forty-eight HIV+ individuals and 17 HIV− control participants were recruited for study participation by the San Diego HIV Neurobehavioral Research Center (HNRC) from surrounding communities. All participants provided written informed consent. Magnetic resonance spectroscopy and imaging were completed at the VA San Diego Healthcare System, whereas medical examinations were done at the HNRC. HIV+ individuals were stratified into groups based on their antiretroviral regimen at study enrollment. Eighteen individuals were taking an antiretroviral regimen that included stavudine (d4T) and/or didanosine (ddI) (HIV+/d*), 14 individuals were taking antiretrovirals that included zidovudine and lamivudine (HIV+/ZDV), and 16 individuals were not taking antiretrovirals at the time of study enrollment (HIV+/naïve). Individuals in the HIV+/ZDV group were not taking stavudine, didanosine, or deoxycytidine. Table 2 displays the antiretroviral regimen for each participant at the time of examination. As presented in Table 3, the HIV+ groups and HIV− controls were comparable on age and levels of education. Each group had a comparable and not statistically different proportion of female and nonwhite participants. In addition, the HIV+ groups had similar estimated lengths of HIV infection, which was based on self-report.

Participants were excluded if they had a history of kidney, lung, heart, or autoimmune disorder whose systemic manifestations might independently influence brain metabolite concentrations. We also excluded anyone meeting criteria for HIV-associated dementia, based on criteria established by the American Academy of Neurology. Participants were also excluded for head injury with loss of consciousness greater than 30 min, penetrating skull wound, brain surgery, seizure disorder, and cerebral palsy. HIV+ participants had no history of CNS opportunistic infection (OI) based on self-report and confirmed with magnetic resonance imaging. Table 2 lists OI history for HIV+ individuals.

Each participant received a comprehensive medical examination. The assessment included a neuromedical history, review of systems, neurological, and general physical examination, brain MRI, blood sample, and in some cases lumbar puncture for cerebrospinal fluid (CSF) analysis. CD4+ lymphocyte counts on examination and CD4 count nadir, based on self-report, are presented in Table 2. HIV+/d*, HIV+/ZDV, and HIV+/naïve participants had similar levels of immune functioning as measured by total CD4 count per milliliter blood. HIV− controls had higher CD4 counts on average than the combined groups of HIV+ participants. HIV RNA assays for plasma and CSF were performed by reverse transcriptase–polymerase chain reaction (RT-PCR) (Roche Amplicor, Branchburg, NJ). HIV+/d* and HIV+/ZDV had significantly less $\log_{10}$ copies of HIV RNA in plasma when compared to HIV+/naïve. In addition, HIV+/d* had significantly less copies of HIV RNA in CSF when compared to HIV+/naïve. HIV+/ZDV and HIV+/naïve did not differ significantly in amount of HIV RNA present in CSF. Blood hemoglobin and serum albumin were used to estimate illness severity. These measures were similar between HIV+ and HIV− individuals. A neuroradiologist evaluated brain MRIs to rule out neurologic complications and opportunistic brain disease that may complicate interpretation of spectroscopic data. No participant was excluded on this basis.

MRS

A frontal lobe white matter and gray matter region were localized. Figure 2 displays representative localizations of the regions of interest (ROI). Spectra were collected using a clinical, General Electric (Fremont, California) 1.5-Tesla scanner (Signa LX 8.×). PRESS acquisition with an echo time of 35 ms and repetition time of 3000 ms provided reliable measures of the resonances associated with *myo*-inositol, choline-containing compounds, creatine + phosphocreatine, and NAA. Sixty-four acquisitions were averaged for each 20-mm^3 ROI. Water suppression was achieved by three successive radiofrequency pulses of fixed length followed by crusher gradients (CHESS) and optimized transmitter gain. The transmitter gain was optimized

Table 2 Antiretroviral regimen, CD4 nadir based on self-report, CD4 count on examination, and history of opportunistic infections for all HIV+ participants

Group	*Antiretroviral regimen*	*CD4 count nadir (cells/ml)*	*CD4 count (cells/ml)*	*Opportunistic infections history*
HIV+/d*				
1	ddI, d4T, abacavir	30	97	PCP, Wasting, MAC
2	d4T, 3TC, nelfinavir	56	101	—
3	ddI, d4T, abacavir	100	208	KS
4	ddI, d4T, AZT, indinavir, ritonavir	224	224	—
5	d4T, 3TC, nelfinavir	220	231	—
6	ddI, d4T, 3TC, indinavir, ritonavir	40	275	PCP
7	d4T, 3TC, efavirenz	156	379	—
8	d4T, abacavir, ritonavir	150	415	—
9	d4T, 3TC, efavirenz	44	419	Cryptosporidium, MAC
10	ddI, AZT, ritonavir, indinavir	250	479	KS
11	d4T, 3TC, abacavir	150	486	—
12	ddI, abacavir, efavirenz	500	552	Wasting
13	ddI, d4T, indinavir	150	576	Recurrent Pneumonia
14	d4T, 3TC, saquinavir, ritonavir	293	590	—
15	d4T, 3TC, nevirapine	250	591	—
16	d4T, abacavir, indinavir, ritonavir	470	684	—
17	d4T, 3TC, indinavir	160	739	Wasting
18	ddI, d4T, nelfinavir, efavirenz	680	1101	—
HIV+/ZDV				
1	AZT, 3TC, nelfinavir, efavirenz	270	NA	—
2	AZT, 3TC, saquinavir-sgc	40	132	—
3	AZT, 3TC, efavirenz	2	167	PCP, Wasting, Candidiasis, MAC
4	AZT, 3TC, nevirapine	63	181	KS
5	AZT, 3TC, nelfinavir, nevirapine	202	202	—
6	AZT, 3TC, ritonavir, indinavir	214	234	—
7	AZT, 3TC	150	314	—
8	AZT, 3TC, indinavir	26	339	PCP
9	AZT, 3TC, nelfinavir	300	437	—
10	AZT, 3TC, ritonavir, indinavir	117	449	—
11	AZT, 3TC, efavirenz	501	460	—
12	AZT, 3TC, saquinavir-sgc	190	557	—
13	AZT, 3TC, nevirapine	600	790	—
14	AZT, 3TC , ritonavir, indinavir	699	922	—
HIV+/naïve				
1	—	2	7	PCP, CMV, Wasting, KS
2	—	0	9	PCP
3	—	15	49	PCP, KS
4	—	117	117	—
5	—	287	339	—
6	—	287	406	—
7	—	426	426	—
8	—	400	445	—
9	—	214	472	—
10	—	450	498	PCP, Wasting
11	—	398	503	—
12	—	274	517	—
13	—	600	653	—
14	—	490	708	—
15	—	NA	861	Not available
16	—	600	990	PCP

ddI = didanosine; d4T = stavudine; saquinavir-sgc = saquinavir-soft gell capsules; AZT = zidovudine; 3TC = lamivudine; PCP = Pneumocystis carinii pneumonia; KS = Kasposi's sarcoma; CMV = cytomegalovirus; MAC = Mycobacterium avium complex; NA = not available.

via minimization of the water signal, and spectra were autoshimmed.

Spectral analysis was completed as reported previously (Schweinsburg *et al*, 2001), using LCModel version 5.2-1 (Provencher, 1993). LCModel analyzes *in vivo* spectra as a linear combination of a basis set of complete model spectra of metabolites *in vitro* (Provencher, 1993). Free-induction decays (FIDs) were zero-filled to double the points and filtered with a finite discrete convolution to account for field inhomogeneities and eddy currents. FIDs were automatically zero- and first-order phase corrected. A representative spectrum is presented in Figure 3.

Absolute concentrations were obtained by scaling the *in vivo* spectrum to the unsuppressed water

Table 3 Means and standard deviations for demographic characteristics, estimated length of HIV infection, and measures of illness severity

	*HIV+/d** (*n* = 18)	*HIV+/ZDV* (*n* = 14)	*HIV+/naïve* (*n* = 16)	*HIV−* (*n* = 17)	*P value*
Age (years)	42.6 (8.7)	37.2 (8.6)	34.9 (8.1)	39.0 (9.4)	.08
Education (years)	14.4 (1.9)	13.5 (2.5)	13.7 (1.5)	14.1 (2.0)	.59
% Female	11.1	21.4	18.8	17.6	.87
% Non-white	33.3	42.9	50	47.1	.77
Length of HIV infection (years)	8.0 (5.5)	7.3 (6.3)	6.9 (5.0), *n* = 15	NA	.86
CD4 count (cells/ml)	452.7 (251.1)	398.9 (243.1), *n* = 13	437.4 (289.1)	706.0 (219.0), *n* = 15	.006[a]
Hemoglobin (gm/dl)	14.4 (1.4)	13.5 (1.0)	13.8 (2.3), *n* = 15	14.0 (1.4)	.45
Albumin (mg/dl)	4.2 (0.3)	4.3 (0.3)	4.0 (0.3), *n* = 14	4.2 (0.3)	.08
Plasma viral load ($\log_{10}$ c/mL)	2.30	2.30	4.26, *n* = 15	NA	.001[b]
CSF viral load ($\log_{10}$ cells/mL)	1.69, *n* = 14	1.69, *n* = 8	2.69, *n* = 14	NA	.01[c]

Note. Medians are presented for measures of plasma and cerebrospinal fluid (CSF) viral load.
[a]HIV− > HIV+/d*, HIV+/ZDV, HIV+/naïve; [b]HIV+/naïve > HIV+/d*, HIV+/ZDV; [c]HIV+/naïve > HIV+/d*.

peak. Metabolites were corrected for CSF content in the ROI. We employed a fast spin echo technique to quantitate CSF in the ROI using a heavily T2-weighted imaging sequence (TE = 512 ms, TR = 9999 ms, ET = 16, FOV = 24 cm, slice thickness = 5 mm interleaved) (Schweinsburg *et al*, 2000). This technique results in bright CSF and black brain tissue, where any high-intensity signal represents bulk water and can be used to determine fraction CSF (fCSF) from pure CSF signal. The pure CSF signal is determined semiautomatically using a histogram obtained from the lateral ventricles. The calculation of fCSF in any ROI then becomes a fraction of the integrated intensity relative to that of pure CSF signal adjusted for volume of the prescribed ROI. Based on these methods, the HIV+ groups and HIV− controls showed comparable amounts of CSF in both the frontal white matter ($F(3, 64) = 1.43$, $P = .24$) and frontal gray matter ($F(3,64) = 1.79$, $P = .16$) regions of interest. LCModel metabolites were normalized to 100% brain tissue per region of interest with the following formula and reported in institutional units: $C = Co^*(1/(1 - FCSF))$, where C = concentration, Co = metabolite concentration from LCModel output, and FCSF = estimated fraction CSF (McLean *et al*, 2000; Schweinsburg *et al*, 2001) This correction ensures that observed changes represent alterations in metabolite concentration rather than between group differences in the proportion of tissue in each region of interest.

Statistical analyses were performed using JMP 3.2.6 (SAS Institute, Inc., Cary, NC) and SPSS 7.5 (SPSS, Inc., Chicago, IL). One-way ANOVA was used to evaluate group differences in metabolite concentrations. All pairwise comparisons (Tukey HSD) were examined to protect alpha level at .05 for analyses of metabolite concentration. We used Pearson Product-Moment correlations to evaluate the relationship between medical variables and concentrations of NAA.

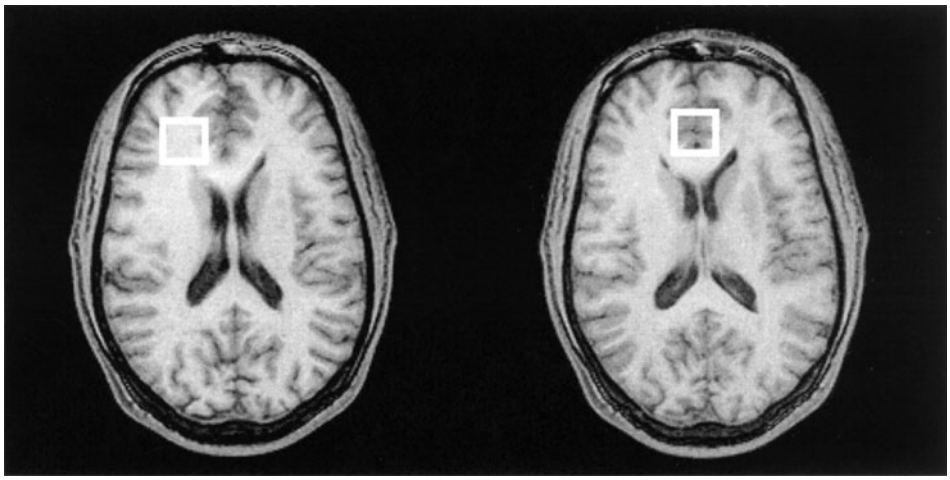

Figure 2 Representative localization of frontal white matter and frontal gray matter regions of interest for a 38-year-old HIV+ individual taking an antiretroviral regimen that included didanosine and stavudine.

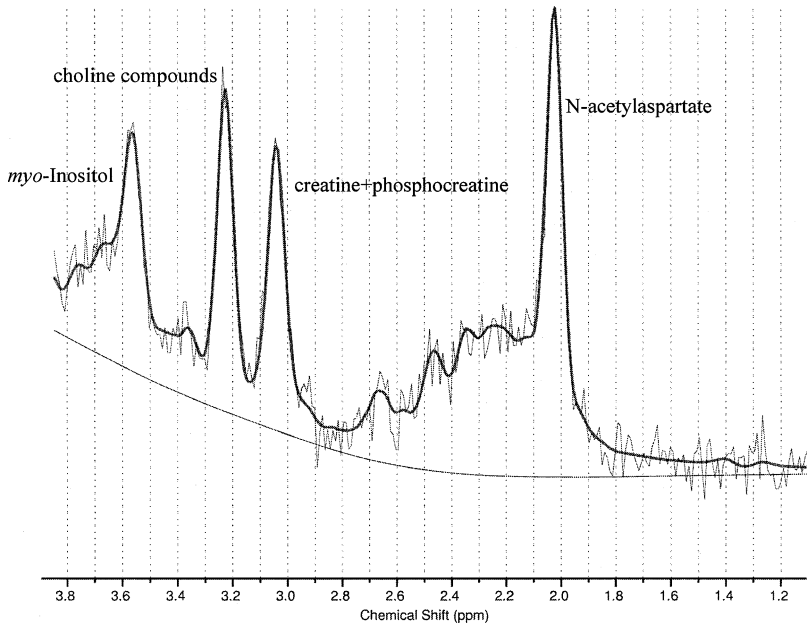

Figure 3 Representative proton MRS spectrum from a frontal white matter region of interest. The bold line represents the LCModel fit for the underlying raw data and the smooth line is the fitted baseline.

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