Published in final edited form as:

J Neurovirol. 2013 June ; 19(3): 209–218. doi:10.1007/s13365-013-0162-1.

Progressive Cerebral Injury in the Setting of Chronic HIV Infection and Antiretroviral Therapy

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Abstract

Background—Emerging evidence suggests that CNS injury and neurocognitive impairment persist in the setting of chronic HIV infection and combination antiretroviral therapy (CART). Yet whether neurological injury can progress in this setting remains uncertain.

Methods—Magnetic resonance spectroscopy, neurocognitive and clinical assessments were performed over two years in 226 HIV-infected individuals on stable CART, including 138 individuals who were neurocognitively asymptomatic (NA). Concentrations of N-acetylaspartate (NAA), creatine (Cr), choline (Cho), myoinositol (MI), and glutamate/glutamine (Glx) were measured in the midfrontal cortex (MFC), frontal white matter (FWM) and basal ganglia (BG). Longitudinal changes in metabolite levels were determined using linear mixed effect models, as were metabolite changes in relation to global neurocognitive function.

Results—HIV-infected subjects showed significant annual decreases in brain metabolite levels in all regions examined, including NAA (2.95%), Cho (2.61%) in the FWM; NAA (1.89%), Cr (1.84%), Cho (2.19%) and Glx (6.05%) in the MFC; and Glx (2.80%) in the BG. Similar

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metabolite decreases were observed in the NA and subclinically impaired subgroups, including subjects with virologic suppression in plasma and CSF. Neurocognitive decline was associated with longitudinal decreases in Glx in the FWM and the BG, and in NAA in the BG.

Conclusions—Widespread progressive changes in the brain, including neuronal injury, occur in chronically HIV-infected persons despite stable antiretroviral treatment and virologic suppression and can lead to neurocognitive declines. The basis for these findings is poorly understood and warrants further study.

BACKGROUND

The successful suppression of HIV infection afforded by combination antiretroviral therapy (CART) has led to a significant decrease in systemic as well as brain complications, including neurocognitive and functional impairment, commonly referred to as AIDS dementia complex (ADC) or HIV-associated neurocognitive disorder (HAND) (Antinori *et al*, 2007; Hammer *et al*, 2006; Navia *et al*, 1986a; Navia *et al*, 1986b; Palella *et al*, 1998; Robertson *et al*, 2007; Sacktor *et al*, 2002). Although dementia has become relatively rare in settings with ready access to CART, milder forms of HAND remain common, with nearly 50% of chronically treated people showing neurocognitive impairment, comparable to rates reported pre-CART (Heaton *et al*, 2010; Heaton *et al*, 2011; Tozzi *et al*, 2007). Furthermore, increasing evidence largely drawn from neuroimaging studies suggest that brain pathology persists in CART-treated individuals (Cardenas *et al*, 2009; Cohen *et al*, 2010a; Cohen *et al*, 2010b; Harezlak *et al*, 2011; Thompson *et al*, 2005). Nonetheless, it remains unclear whether brain injury can further progress in the setting of stable disease and treatment, particularly among neurocognitively asymptomatic individuals.

Proton magnetic resonance spectroscopy (MRS) provides a sensitive non-invasive method to measure specific cerebral metabolites that reflect neuronal and glial dysfunction and neuroinflammation. Numerous cross-sectional studies in HIV-infected persons and in SIV macaque model have described a consistent pattern of metabolite abnormalities in both grey and white matter brain regions, particularly in the frontal white matter and basal ganglia, comprising of alterations in N-acetylaspartate (NAA), a marker of neuronal metabolism, and choline-containing compounds (Cho) and myoinositol (MI), markers of cell membrane damage and glial cell activity (Chang *et al*, 2004; Harezlak *et al*, 2011; Lentz *et al*, 2009; Lopez-Villegas *et al*, 1997; Meyerhoff *et al*, 1999; Mohamed *et al*, 2010; Paul *et al*, 2007; Tracey *et al*, 1996; Tracey *et al*, 1997; Urenjak *et al*, 1993; Yiannoutsos *et al*, 2004). Metabolite abnormalities have also been associated with disease history, neurocognitive function, and volumetric brain abnormalities (Chang *et al*, 2004; Cohen *et al*, 2010a; Harezlak *et al*, 2011; Mohamed *et al*, 2010; Paul *et al*, 2007; Yiannoutsos *et al*, 2004).

Despite considerable evidence of HIV-associated brain disease, there have been no published longitudinal studies to date of metabolite abnormalities in the setting of chronic infection and treatment. The HIV Neuroimaging Consortium (HIVNC) was formed to examine such changes in a prospective multicenter cohort of chronically HIV-infected individuals with history of advanced disease (nadir CD4 < 200 cells/mL) on stable CART. In a recent cross-sectional MRS study of 240 HIV-infected individuals, we reported significant elevations in Cho and MI in all groups, while decreased NAA levels were observed only among those with neurocognitive impairment (Harezlak *et al*, 2011). The current prospective MRS and neurocognitive study was performed in 226 of these individuals, including 138 neurocognitively asymptomatic individuals, over two years. We hypothesized that, despite stable antiretroviral treatment, such individuals will show progressive changes in cerebral metabolite levels, even in the setting of virologic suppression. In addition, we hypothesized that such changes will be associated with neurocognitive decline.

METHODS

Participants

Participants included 226 HIV-infected individuals who were enrolled in a longitudinal study of HIV-associated brain injury at the following sites: University of California San Diego, University of California Los Angeles (UCLA), Harbor-UCLA Medical Center, Stanford University, University of Colorado, University of Pittsburgh, and Rochester University. Demographic and clinical characteristics are shown in Table 1. All participants gave written informed consent, and institutional review boards at each site approved the protocol. Inclusion criteria included: nadir CD4 level < 200 cells/ml, stable FDA-approved antiretroviral regimen 12 consecutive weeks; hemoglobin > 9.0 gm/dl; serum creatinine 3x upper limit of normal (ULN); AST (SGOT), ALT (SGPT), and alkaline phosphatase 3xULN. Exclusion criteria included: neurologic conditions including seizure disorder, stroke, head trauma with loss of consciousness > 30 minutes, multiple sclerosis, brain infection apart from HIV, and brain neoplasms; history of severe psychiatric disorders; active alcohol and substance abuse within 6 months of study; diabetes mellitus with a fasting glucose > 140 mg/dl.

Clinical Assessments

Participants underwent repeated neuroimaging, neurological, and neuropsychological assessments as previously described (Harezlak *et al*, 2011). Neuropsychological evaluation was performed in cognitive domains most implicated in HIV infection, including processing speed, verbal fluency, executive functioning, working memory, learning and memory, and psychomotor function (Heaton *et al*, 1995). Individual tests in each cognitive domain are listed in Table 2. The most updated demographically corrected norms for each test were used to convert individual test scores into standard scores (T scores, mean = 50, SD = 10). A previously validated algorithm was used to derive a Global Deficit Score (GDS) for each participant reflecting the overall degree of cognitive impairment. Specifically, the demographically corrected T scores on individual neuropsychological measures were converted to deficit scores ranging from 0 (no impairment) to 5 (severe impairment). The GDS was then derived by averaging the deficit scores for all measures. Previous studies have demonstrated the utility of the GDS in detecting cognitive impairment in HIV-infected individuals (Carey *et al*, 2004; Heaton *et al*, 1995).

AIDS dementia complex (ADC) staging was performed by a trained clinician at each site and was based on the neurological examination, assessment of functional impairment, and neuropsychological performance as previously described (Harezlak *et al*, 2011). Subsequent to the inception of this study, a new nosologic classification for HAND was proposed, with ADC stage 0 (asymptomatic) corresponding to neurocognitively normal, stage 0.5 (subclinical) to asymptomatic neurocognitive impairment (ANI), stage 1 (symptomatic) corresponding to mild neurocognitive disorder (MND), stage 2 or greater to HIV-associated dementia (HAD) (Antinori *et al*, 2007).

MRS Acquisition and Processing

¹H MRS spectra were acquired with 1.5-tesla General Electric magnetic resonance systems (GE Healthcare, Waukesha, WI) at 7 participating sites using the GE quadrature head coil and the manufacturer's PRESS sequence. Field homogeneity and water suppression were adjusted using automated algorithms from GE. Water suppressed spectra were collected with TE/TR = 35/3000ms, bandwidth = 2500 Hz, 128 averages. In addition, a customized pulse program was run to automatically collect single-scan, fully relaxed water FIDs from each voxel at 7 different echo times. (TE = 35, 45, 65, 100, 200, 500 and 1500 ms; TR = 15 s). From this, voxel partial volumes were determined to correct absolute metabolite

concentrations (Ernst *et al*, 1993; Kreis *et al*, 1993). Regions of interest (ROI) 6 cc in volume were prescribed in the midfrontal cortical gray matter located in the anterior cingulate (MFC), frontal white matter located in the centrum semiovale (FWM), and basal ganglia (BG). Laterality of the FWM and BG ROIs were alternated between participants but maintained for repeat studies for each participant.

For quality control, sites were required to acquire a phantom spectrum within 24 hours of each in vivo scan, using the identical MRS protocol as described above. The quality of the MR spectra and voxel locations were monitored continuously University of Hawaii, based on visual inspection and outputs from the LC Model spectral analysis software (Provencher, 2001), with unsuppressed water FID at TE = 35ms used for eddy-current correction. The %SD (estimated standard deviations as percent of the estimated concentrations) and FWHM (full width at half maximum, an estimate of peak width) were used to determine spectrum quality. %SD of less than 20 and FWHM of less than 0.1 ppm constitute a reliable estimate for a particular metabolite. Both absolute concentration and metabolite/Cr ratio were computed for N-acetylaspartate (NAA), myoinositol (MI), choline-containing compounds (Cho), glutamate+glutamine (Glx), and creatine (Cr). The coefficient of variation of the metabolite estimates were <15%. Analysis of the phantom data from the current study showed that the variability in measurements of metabolite ratios was smaller than the variability in the study population, consistent with our reported findings in an independent cohort (Lee *et al*, 2003). The variability in the phantom metabolite ratio between the seven sites did not exceed 7% from the overall mean with most of the variations falling within 2-3%. Longitudinal analysis of phantom measurements taken at all sites also showed limited variability in metabolite concentrations over time.

Statistical Analysis

Longitudinal analyses were performed using linear mixed effect models with length of time since enrollment as an independent covariate and participant-specific random intercepts and slopes (Laird and Ware, 1982). The annual rates of change and their associated standard errors were estimated based on the regression coefficients corresponding to the mean metabolite levels at baseline and the change in their concentrations over the 2-year time period. Additional longitudinal analyses were done for participants with undetectable plasma HIV RNA (N = 184) as well as undetectable CSF HIV RNA at baseline (N = 51). In addition, we determined the rates of change for each clinical group defined by baseline ADC stages 0, 0.5, and 1+ (see Clinical Assessments section above). To determine disease stability, the longitudinal change in CD4 count and the proportion of subjects with detectable plasma HIV RNA levels were assessed by linear and generalized linear mixed models, respectively. Linear mixed methods analyses were also used to determine the relationships between the rates of changes in metabolite levels and changes in global neurocognitive function. First, we predicted the participant-specific rates of both metabolite change and neurocognitive change using individual random intercepts and slopes. Then the Pearson's product moment correlations were estimated between the individual predicted metabolite rates and neurocognitive score rates, and tested for the difference of these correlations from zero using Fisher's Z transform.

RESULTS

Cohort

Median age of participants was 47 years, while median duration of HIV infection was 11 years at baseline. All participants had history of advanced immunosuppression (median nadir CD4 = 37 cells/ml), but a median current CD4 level of 326, consistent with successful immunological restoration in response to antiretroviral therapies. The majority of

participants were maintained on the same CART regimen for at least 1.5 years, with 81% showing undetectable plasma HIV RNA levels. CSF HIV RNA levels were available for 82 participants, of which 60 (73%) were undetectable; while both plasma and CSF HIV RNA were undetectable in 51 participants (62%). Longitudinal analyses examining HIV disease stability during the study showed that mean CD4 levels increased by 6.9% over two years, while the percentage of patients with undetectable plasma RNA remained stable at around 80%.

Longitudinal Metabolite Changes in the Whole Cohort

Significant decreases in absolute concentrations were observed in all brain regions examined. The FWM showed significant annual decreases in NAA (2.95%, $p < .0001$) and Cho (2.61%, $p = .0001$). The MFC also showed significant decreases in NAA (1.89%, $p = .0007$), Cr (1.84%, $p = .0059$), Cho (2.19%, $p = .0123$), and Glx (6.05%, $p < .0001$). A marginal decrease in MI was found in the MFC (1.81%, $p = .0578$). The BG showed a significant decrease in only Glx (2.80%, $p = .0043$). Analyses of metabolite/Cr ratios showed similar patterns of change in fewer brain regions. Significant decreases in NAA/Cr (2.51%, $p < .0001$) and Cho/Cr (1.74%, $p = .0123$) were observed in the FWM, while the MFC showed decreases in Glx/Cr (3.84%, $p < .0001$). No significant changes in metabolite/Cr ratios in the BG were found. Table 4 shows estimated annual changes in all measured absolute metabolite concentrations and metabolite/Cr ratios in the three brain regions.

The estimated rates of change in the phantom metabolite levels were much smaller than those found in the study cohort. Specifically, the rates of change in absolute concentrations were -1.10% for NAA, -0.93% for Cr, and -1.18% for Cho, while rates of change observed in the study cohort were between 1.7 and 2.7 times larger in magnitude for metabolites showing significant changes. Similarly, the rates of change in the relative concentrations were -0.37% for NAA/Cr and -0.63% for Cho/Cr ratio. Again, the significant changes in the metabolite ratios were between 2.8 and 6.8 times larger in magnitude in the cohort.

Longitudinal Changes by Neurocognitive Status

To examine whether metabolite changes differed with neurocognitive status, longitudinal analyses of metabolite levels were performed in subgroups stratified according to ADC stage (Table 4). Overall, NA (ADC 0) and subclinically impaired (ADC 0.5) subjects showed similar patterns of metabolite changes, including significant decreases in NAA and Cho in the FWM, and decrease in Glx in the MFC. In addition, NA subjects showed significant decreases in NAA and Cr in the MFC, while participants in the subclinical group showed significant decreases in Cho in the MFC and Glx in the BG. Symptomatic subjects (ADC stage 1) showed relatively fewer metabolite changes, including significant decreases in Glx in the MFC, in addition to decreases in Cr and Cho in the BG.

Longitudinal Changes in Participants with Undetectable HIV RNA

To further examine whether significant metabolite changes occurred even in virologically suppressed individuals, we examined longitudinal metabolite changes in a subgroup of subjects with undetectable plasma HIV RNA at baseline (N=184) and another subgroup with undetectable HIV RNA in both plasma and CSF (N=51) (Table 4). Participants with plasma virologic suppression showed similar patterns of metabolite changes as in the whole cohort. In participants with virologic suppression in both plasma and CSF (N = 51), significant decreases were found in NAA in the FWM, Glx and MI in the MFC, and NAA in the BG.

Changes in Metabolite Levels Relative to Cognitive Decline

We assessed whether changes in metabolite levels were associated with changes in global cognitive function. Increase in the GDS score (reflecting cognitive decline) was significantly associated with decreases in Glx/Cr in the FWM ($p = .0319$) and Glx/Cr in the BG ($p = .0468$). A marginal relationship was found between increased GDS and decreased absolute NAA level in the BG ($p = .0568$).

DISCUSSION

Whether chronically HIV-infected individuals can develop progressive brain abnormalities despite successful viral suppression and immunological restoration in response to antiretroviral treatment has remained an important yet unresolved question. In this prospective study, we found significant annual decreases ranging from 2% to 6% in the levels of several metabolites, notably NAA and Glx, markers of neuronal and glial cell function, in both cortical and subcortical brain regions. The current results are remarkable in that progressive metabolite changes were observed even among neuroasymptomatic individuals with virologic suppression. Together these findings suggest that that HIV-associated brain injury can progress in the absence of overt symptoms, and that effective antiretroviral treatment may not be sufficient to prevent this complication.

There have been few MRS prospective studies reported (Kantarci *et al*, 2007; Olson *et al*, 2008; Pilatus *et al*, 2009; Schott *et al*, 2010). Metabolite levels in healthy controls acquired using similar methods as described here remain relatively unchanged over time compared to those observed in the study patient. In this study, longitudinal phantom measurements were acquired with each imaging session to assess whether changes observed in the HIV subjects could be attributed to scanner drift or measurement instability. The findings were consistent with those reported in previous studies among healthy adults and argue strongly that the observed changes were due to biological rather than scanner-related factors.

The current findings expand on a large body of cross sectional evidence showing the association between HIV infection and various patterns of brain injury as measured by MRS (Chang *et al*, 2004; Lopez-Villegas *et al*, 1997; Meyerhoff *et al*, 1999; Mohamed *et al*, 2010; Paul *et al*, 2007; Tracey *et al*, 1996; Urenjak *et al*, 1993; Yiannoutsos *et al*, 2004). Observed abnormalities have consistently included elevated levels of the inflammatory markers Cho and MI among HIV-infected individuals regardless of cognitive status whereas decrease in NAA, a marker of neuronal integrity, has consistently been observed in individuals with NCI, suggesting that neural dysfunction may be necessary in its expression (Chang *et al*, 2004; Harezlak *et al*, 2011; Mohamed *et al*, 2010; Paul *et al*, 2007; Yiannoutsos *et al*, 2004). It is of interest then that, in the setting of chronic infection and treatment, progressive decreases in NAA both in grey and white matter were observed in NA subjects suggesting these individuals may be at risk for developing NCI. In contrast symptomatic subjects (ADC>1) although showing similar patterns of change, had more extensive involvement of the basal ganglia, consistent with earlier observations that injury to this region is a critical event leading to symptomatic impairment (Brew *et al*, 1995; Navia *et al*, 1986a; Rostasy *et al*, 1999). Further, the prominent changes in the cortical grey matter across the groups are consistent with recent reports of persistent cortical atrophy in HIV-infected people (Cohen *et al*, 2010a; Cohen *et al*, 2010b; Thompson *et al*, 2005), and suggest that the pattern of HIV-associated brain dysfunction in the context of treatment may have evolved from what was previously considered a subcortical disorder (Heaton *et al*, 1995; Navia *et al*, 1986a; Navia *et al*, 1986b).

Decreases in Glx comprised the largest metabolite change observed across the clinical groups, particularly in the MFC and BG. The Glx peak represents the levels of both

glutamine and glutamate and may change in response to events affecting glial and neuronal function (Urenjak *et al*, 1993). Recent cross-sectional studies in HIV have found significant decreases in Glx even in NA individuals (Harezlak *et al*, 2011; Mohamed *et al*, 2010). Changes in Glx may thus represent a critical event that occurs prior to the onset of neurocognitive symptoms, and may offer a sensitive biomarker of neural health in the early stages of infection. Together with NAA, these metabolites may provide a useful approach to monitoring cerebral function over the course of HIV disease in the era of CART.

The observed declines in Cho, a marker of membrane turnover, were unexpected as was the relative absence of changes in MI, a measure of cellular osmotic changes and glial cell proliferation (Lopez-Villegas *et al*, 1997; Meyerhoff *et al*, 1999; Tracey *et al*, 1996; Urenjak *et al*, 1993). In contrast to the findings from cross sectional studies, this observation suggests these two metabolites can in fact dissociate, possibly as a consequence of the different cellular processes they reflect. Combined, these results suggest a pattern of continuing neural injury in the setting of stable brain inflammation. The reason for this is not presently known but it is possible that CART may be effective in stabilizing inflammatory responses while other neuroactive events remain unchecked, resulting in membrane damage with loss of choline containing compounds. Quantitative pathological studies are needed to confirm this model. The decrease in creatine, generally considered a marker of energy metabolism, was found only in MFC and BG, suggesting these regions may be particularly vulnerable to global disturbances in metabolic function.

This is the first study to show associations between longitudinal changes in cerebral metabolites and cognitive decline, and expands on a small number of cross-sectional findings (Mohamed *et al*, 2010; Paul *et al*, 2007). Notably, global cognitive decline was associated with decreases in Glx levels in the frontal white matter and the basal ganglia, and marginally with decrease in NAA in the basal ganglia. The Glx finding is particularly noteworthy, as the role of this metabolite in cognitive function has remained uncertain. The current findings also extend a recent study showing significant associations of Glx levels with executive function, working memory, and motor impairments, and together suggests decreases in Glx may contribute importantly to HIV-associated neurocognitive impairment (Mohamed *et al*, 2010).

The basis for the current findings is unclear but may be due to the effects of one or more host or disease-related factors. Several studies pre-CART had identified plasma and CSF HIV RNA levels in addition to low current CD4 as important risk factors for neurocognitive impairment (Ellis *et al*, 1997; McArthur *et al*, 1997). More recently, several groups have reported significant associations between nadir CD4, brain atrophy and neurocognitive impairment (Cohen *et al*, 2010b; Heaton *et al*, 2011). However, preliminary findings suggest that mechanisms contributing to progressive brain injury may involve complex relationships among several factors, including HIV viral load, CD4 levels, duration of infection and of treatment, and aging. We have recently shown that the chemokines, MCP-1 and IP10, are associated with decreases in NAA suggesting that markers of immune activation and inflammation contribute to neuropathogenesis (Letendre *et al*, 2011). In addition, underlying comorbid disorders such as hepatitis C, diabetes mellitus, obesity, possibly mediated through one or more inflammatory factors, may also affect risk (Valcour *et al*, 2004; Valcour *et al*, 2005; van Gorp and Hinkin, 2005). Further studies are needed to disentangle the effects of these factors and their relationships to varying patterns of brain injury.

Results from the current study indicate that progressive brain injury continues to occur in medically stable HIV-infected persons on effective antiretroviral therapies, and these changes are related to declines in cognitive function over time. These results further support MRS as a sensitive measure of HIV-associated brain injury even in neurocognitively

asymptomatic individuals, and points to its potential value, in combination with cognitive assessments, in monitoring disease progression and response to treatment. These findings suggest that careful monitoring of neurological function in HIV-infected persons is warranted in the setting of chronic disease and treatment.

Acknowledgments

Sources: NS36524, NS38841, RR025780, U01MH083506, R01NS036524, AI069424, MH083550, AA020235

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Figure 1.

Longitudinal changes in absolute concentrations of NAA, Cho, Cr, and Glx in the midfrontal cortex (MFC). Each thin grey line represents the metabolite concentration level for each participant at all observed time points, while the red solid line gives the estimate of the mean metabolite population trajectory and broken lines give 95% pointwise confidence intervals of the estimated mean.

Table 1

Demographic and relevant clinical information.

	ADC Stage			Total
	0	0.5	1+	
N	138	48	40	226
Age (years)	46 (41, 52)	49 (44, 52)	49 (43, 58)	47 (41, 52)
Gender (% male)	112 (81%)	40 (83%)	36 (90%)	188 (83%)
Ethnicity (% Caucasian)	100 (72%)	27 (56%)	30 (75%)	157 (69%)
College education or higher	84 (61%)	27 (56%)	27 (68%)	138 (61%)
CD4 count (cells/ml)	327 (196, 495)	330 (249, 428)	307 (194, 466)	326 (196, 495)
Nadir CD4 (cells/ml)	40 (13, 94)	36 (15, 69)	38 (12, 116)	37 (13, 94)
Plasma viral load (% undetectable)	117 (85%)	37 (77%)	30 (75%)	184 (81%)
CSF viral load (% undetectable) *	26 (63%)	12 (75%)	22 (88%)	60/82 (73%)
HIV infection duration (years)	12 (5, 17)	8.5 (5.75, 16)	12 (9.5, 17.5)	11.5 (5, 17)
CART treatment duration (years)	1.53 (0.89, 2.90)	1.45 (0.57, 2.82)	1.58 (0.73, 3.52)	1.53 (0.89, 2.90)

* CSF viral load data were available for 82 participants.

Note: Values reported as median (Q1, Q3) unless noted otherwise. CART = combination antiretroviral therapy. No significant group differences were found in any of the variables ($p > .05$).

Table 2

Neuropsychological tests administered as part of this study organized by cognitive domains. Details of the individual tests have been described elsewhere (Heaton *et al*, 1995; Rippeth *et al*, 2004).

Cognitive Domains	Tests
Processing Speed	WAIS-III Digit Symbol WAIS-III Symbol Search Trail Making: Part A
Verbal Fluency	Controlled Oral Word Association (FAS) - Correct Words Animal Naming - Correct Words Action Fluency - Correct Words
Executive Function	Trail Making: Part B Stroop Test
Working Memory	Paced Auditory Serial Addition Task WAIS-III Letter Number Sequencing
Learning	Hopkins Verbal Learning Test - Immediate Recall Benton Visual Memory Test - Immediate Recall
Memory	Hopkins Verbal Learning Test - Delayed Recall Benton Visual Memory Test - Delayed Recall
Psychomotor	Grooved Pegboard

Table 3

Estimated annual changes in metabolite concentrations for all 226 participants. Significant changes are highlighted in grey. Results are displayed for all participants, and further stratified by AIDS Dementia Complex (ADC) staging.

Region	Metabolite	All (N=226)		ADC Stage			
		0 (N=138)		0.5 (N=48)		1 (N=40)	
		Rate	p-value	Rate	p-value	Rate	p-value
<u>Absolute Concentrations</u>							
FWM	NAA	-2.95%	0.0000	-3.22%	0.0000	-3.59%	0.0002
	Cr	-0.43%	0.4787	-0.74%	0.3277	0.00%	0.9981
	Cho	-2.61%	0.0001	-2.72%	0.0013	-2.99%	0.0449
	MI	-1.38%	0.1194	-1.69%	0.1228	-1.92%	0.3265
	Glx	-1.90%	0.0516	-1.77%	0.1521	-0.62%	0.7862
MFC	NAA	-1.89%	0.0007	-1.57%	0.0154	-2.34%	0.0522
	Cr	-1.84%	0.0059	-1.92%	0.0153	-2.04%	0.1713
	Cho	-2.19%	0.0123	-1.22%	0.2705	-4.36%	0.0179
	MI	-1.81%	0.0578	-0.83%	0.4880	-3.34%	0.1317
	Glx	-6.05%	0.0000	-5.33%	0.0000	-6.82%	0.0001
BG	NAA	-0.41%	0.5245	-0.51%	0.4958	1.00%	0.4578
	Cr	-0.35%	0.6103	0.09%	0.9150	1.56%	0.2216
	Cho	-0.60%	0.4653	-0.03%	0.9778	1.81%	0.3052
	MI	-0.34%	0.7423	-0.54%	0.6657	2.06%	0.3515
	Glx	-2.80%	0.0043	-1.75%	0.1594	-5.89%	0.0083
<u>Metabolite/Cr Ratios</u>							
FWM	NAA/Cr	-2.51%	0.0000	-2.68%	0.0000	-3.68%	0.0011
	Cho/Cr	-1.74%	0.0123	-1.64%	0.0685	-2.73%	0.0919
	MI/Cr	-0.81%	0.3638	-0.08%	0.9403	-1.65%	0.3955
	Glx/Cr	-0.72%	0.4668	-0.61%	0.6342	-0.67%	0.7650
	NAA/Cr	-0.13%	0.8081	-0.12%	0.8474	-0.19%	0.8710
MFC	Cho/Cr	-0.59%	0.3956	0.31%	0.7414	-2.68%	0.0466
	MI/Cr	-0.87%	0.2761	-0.08%	0.9361	-1.84%	0.2960
	Glx/Cr	-3.84%	0.0000	-3.06%	0.0020	-4.84%	0.0067
						-5.28%	0.0225

Region	Metabolite	All (N=226)						ADC Stage					
		0 (N=138)			0.5 (N=48)			1 (N=40)					
		Rate	p-value	Rate	p-value	Rate	p-value	Rate	p-value	Rate	p-value	Rate	p-value
BG	NAA/Cr	0.17%	0.8244	0.23%	0.8129	-0.87%	0.5768	2.42%	0.2425				
	Cho/Cr	-0.64%	0.3789	0.01%	0.9888	-0.51%	0.7616	-2.96%	0.0488				
	MI/Cr	-0.75%	0.4850	-1.30%	0.3052	-0.90%	0.6845	1.79%	0.6134				
	Glx/Cr	-1.63%	0.0923	-1.23%	0.2888	-6.33%	0.0012	1.23%	0.6924				

Note: NAA = N-acetylaspartate, Cho = choline-containing compounds, Glx = glutamate+glutamine, Cr = creatine, FWM = frontal white matter, MFC = midfrontal cortical grey matter, BG = basal ganglia.

Estimated annual changes in metabolite concentrations for participants with undetectable HIV RNA in plasma (N=184) and in both plasma and CSF (N=51). Significant changes are highlighted in grey.

Region	Metabolite	Undetectable Plasma HIV RNA (N=184)			Undetectable Plasma and CSF HIV RNA (N=51)*		
		Rate	p-value	Rate	p-value	Rate	p-value
<u>Absolute Concentrations</u>							
FWM	NAA	-2.51%	0.0000			-4.38%	0.0006
	Cr	0.52%	0.4248			-1.12%	0.3591
	Cho	-1.97%	0.0083			-1.70%	0.3129
	MI	-1.11%	0.2392			-0.57%	0.7618
	Glx	-1.71%	0.1129			1.42%	0.5318
MFC	NAA	-1.48%	0.0099			-2.37%	0.0980
	Cr	-1.11%	0.1070			-1.56%	0.3101
	Cho	-2.09%	0.0248			-1.79%	0.3723
	MI	-1.59%	0.0937			-3.10%	0.1649
	Glx	-6.48%	0.0000			-5.48%	0.0004
BG	NAA	-0.22%	0.7447			1.21%	0.4550
	Cr	-0.68%	0.3556			-2.27%	0.1842
	Cho	-0.85%	0.3227			-1.29%	0.5129
	MI	-0.51%	0.6411			3.44%	0.1941
	Glx	-2.85%	0.0076			-1.81%	0.3094
<u>Metabolite/Cr Ratios</u>							
FWM	NAA/Cr	-2.90%	0.0000			-2.63%	0.0166
	Cho/Cr	-1.94%	0.0103			0.08%	0.9487
	MI/Cr	-1.30%	0.1721			1.10%	0.5375
	Glx/Cr	-1.94%	0.0714			3.19%	0.1680
MFC	NAA/Cr	-0.54%	0.3332			-0.62%	0.5545
	Cho/Cr	-0.96%	0.1859			-0.09%	0.9502
	MI/Cr	-1.34%	0.1009			-3.59%	0.0273
BG	Glx/Cr	-4.99%	0.0000			-3.71%	0.0300
	NAA/Cr	0.40%	0.6313			3.21%	0.0441

Region	Metabolite	Undetectable Plasma HIV RNA (N=184)		Undetectable Plasma and CSF HIV RNA (N=51)*	
		Rate	p-value	Rate	p-value
	Cho/Cr	-0.43%	0.5798	0.34%	0.8076
	MI/Cr	-0.54%	0.6446	3.89%	0.1585
	Glx/Cr	-1.57%	0.1317	2.20%	0.3286

* CSF viral load data were available for 82 participants.

Note: NAA = N-acetylaspartate, Cho = choline-containing compounds, Glx = glutamate+glutamine, Cr = creatine, FWM = frontal white matter, MFC = midfrontal cortical grey matter, BG = basal ganglia.