Neuronal-glia markers by Magnetic Resonance Spectroscopy in HIV Before and After Combination Antiretroviral Therapy

Napapon Sailasuta, PhD[1], **Jintanat Ananworanich, MD, PhD**[2,3,4], **Sukalaya Lerdlum, MD**[5], **Pasiri Sithinamsuwan, MD**[6], **James LK Fletcher, BM BCH**[4], **Somporn Tipsuk, BNS**[4], **Mantana Pothisri, BSc**[5], **Tanate Jadwattanakul, MD**[7], **Supunnee Jirajariyavej, MD**[8], **Thep Chalermchai, MD, PhD**[4], **Stephanie Catella, PsyD**[9], **Edgar Busovaca, BA**[9], **Akash Desai, BS**[9], **Robert Paul, PhD**[10], and **Victor Valcour, MD, PhD**[9] on behalf of the SEARCH Study Group

[1]Department of Tropical Medicine, University of Hawaii, Honolulu, HI [2]U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA [3]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, USA [4]SEARCH, Thai Red Cross AIDS Research Center, Bangkok, Thailand [5]Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand [6]Division of Neurology, Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand [7]Queen Savang Vadhana Memorial Hospital, Chomburi, Thailand [8]Taksin Hospital, Bangkok, Thailand [9]Memory and Aging Center, Department of Neurology, University of California, San Francisco, CA, USA [10]Missouri Institute of Mental Health, Department of Psychology, University of Missouri, St. Louis, MO, USA

Abstract

Objective—Combination antiretroviral therapy (cART) can suppress plasma HIV RNA to undetectable levels; yet reports indicate persistent HIV-associated neurocognitive disorders (HAND) among treated individuals. We sought to investigate imaging correlates of incomplete cognitive recovery among individuals with chronic HIV.

Methods—We used single voxel proton magnetic resonance spectroscopy (MRS) in four brain regions to measure changes in neuronal and glia biomarkers in cART-naïve subjects before (n=59, 27 with HAND) and after 12 months of cART.

Results—At baseline we observed elevated total choline (CHO) in the basal ganglia (BG, p=0.002) and in the posterior cingulate gyrus (PCG, p=0.022) associated with HIV-infection. Myo-inositol (MI) was elevated in the frontal white matter (FWM, p=0.040). N-acetylaspartate (NAA) was elevated in the BG (p=0.047). Using a mixed model approach among all HIV-infected individuals at 6 months, we observed decreased NAA in FWM (p =0.031), decreased creatine (CR) in PCG (p=0.026) and increased MI in FGM (p=0.023). At 12 months, we observed an

Corresponding author: Victor Valcour MD PhD, Room 192C, Memory and Aging Center, Sandler Neurosciences Center, UCSF, 675 Nelson Rising Lane, San Francisco, CA 94158. Fax: (415) 476-0213. Phone: (415) 476-3746. vvalcour@memory.ucsf.edu.

increase in BG MI (p=0.038) and in FGM (p=0.021). Compared to those with normal cognition, HAND cases had higher FGM MI (p=0.014) at baseline. At 12 months, individuals that remained cognitively impaired compared to those without HAND exhibited elevated CHO in the PCG (p=0.018) and decreased GLU in both FWM (p=0.027) and BG (p=0.013).

Conclusions—cART started during chronic HIV is associated with reduced neuronal-glia and inflammatory markers. Alterations in CHO are noted among individuals who remain impaired after 12 months of cART.

Keywords

HIV; Magnetic Resonance Spectroscopy; Cognitive Disorders

INTRODUCTION

Central nervous system (CNS) dysfunction is a common characteristic of human immunodeficiency virus (HIV) infection. The underlying neuropathology is largely related to the indirect consequence of disrupted glial function since HIV does not substantially infect neurons. Instead, brain macrophages, microglia, and multinucleated giant cells become infected and instigate a reactive astrocytosis.[1] Subsequent brain injury likely ensues from both toxic HIV particles and toxins from activated macrophages and astrocytes resulting in cognitive impairment as evidenced by neuropsychological testing abnormalities. Even in the era of combination antiretroviral therapy (cART) the prevalence of HIV-associated neurocognitive disorders (HAND) remains substantial.[2–4] Whether these chronic deficits are due to the same mechanisms as seen in untreated HIV is incompletely understood and may be informed by non-invasive measures of glial function and CNS inflammation, as is possible with proton magnetic resonance spectroscopy (MRS).

Abnormal brain chemical concentrations in the basal ganglia (BG), frontal white matter (FWM), frontal gray matter (FGM) and the posterior cingulate gyrus (PCG) have been described in HIV.[5–8] Clinical studies demonstrate abnormal axonal and neuronal markers in HIV-infected individuals with cognitive impairment and other neurological symptoms.[9–11] Our earlier prospective study of the same cART-naïve chronically HIV-infected Thai subjects examined in the current work revealed associations between reservoir levels of HIV DNA in peripheral blood mononuclear cells (PBMC) enriched with those having the $CD14^+$ cell surface marker (monocytes) and MRS abnormalities.[12] In the current study, we examine MRS abnormalities associated with HIV in cART-naïve subjects and those associated with HAND. We also examined changes in MRS that were associated with cART initiation over 12 months in order to assess if MRS abnormalities could be identified in association with continued HAND despite 12 months of continuous cART.

MATERIALS AND METHODS

Participants

HIV-infected adults were enrolled at the SEARCH clinic of the Thai Red Cross AIDS Research Center in Bangkok, Thailand (protocol SEARCH 011, www.ClinicalTrials.gov NCT00782808); details are previously described.[12] All met Thai Ministry Public Health

criteria for initiating cART (CD4 count < 350 cells/mm^3 or symptomatic disease). We enrolled participants using a stratified scheme for PBMC HIV DNA ($>/<$ 1000 copies of HIV DNA/10^6 cells) and age ($>/<$ 35 years), based on a predetermined blinded randomization scheme.[13] HIV-uninfected adults were recruited in a separate normative imaging study (SEARCH 009) capturing one-time MRS data on 28 controls. HIV-infected individuals (n=59) received baseline, 6 months, and 12 months MRS, of whom 51 completed all three visits. All provided written consents approved by the Institutional Review Boards at the Chulalongkorn University in Bangkok and the University of California, San Francisco.

Cognitive assessments

HIV-infected participants completed a 60-minute battery of neuropsychological tests that included the WHO-UCLA Auditory Verbal Learning Task, Color Trails 1 and 2, Digit Symbol Modalities Test, Block Design Tasks, Grooved Pegboard for both hands, finger tapping for both hands, Timed Gait, two verbal fluency tasks (first names and animals) and the Trail Making Test A.[14] We conducted consensus conferences with two of the authors (VV, RP) and a US neurologist to assign HAND diagnoses using 2007 Frascati criteria.[15] We calculated a composite neuropsychological testing score (NPZ- global) as the arithmetic mean of age- and education-adjusted z-scores for performance on individual tests using normative data from over 500 Thai controls[16].

Brain proton MRS

All completed axial 3D T1-weighted spoiled gradient echo MRI (TE = 7ms, TR = 11.2ms, 1mm resolution) on the same 1.5T GE MRI scanner using the same software throughout the study duration (GE Healthcare, software v12.0). An 8-channel head coil and standard body coil was used. Eight cubic centimeters voxels were placed in the normal appearing brain regions in the right BG and FWM, midline frontal gray matter (FGM) and PCG using a double spin echo data acquisition (Probe-p) (TE/TR=35/1500, number of excitations of 128 for FWM, FGM and PCG and 192 for the BG).

Voxels were carefully placed by an experienced technologist (MP) by visual inspection of the MRI screen shot of the prescription as closely monitored by our physicist (NS). The average voxel placement was consistent throughout the study with approximately 4.0 ± 0.5 mm differences between baseline, 6 and 12-month follow-up. We employed commercially available time domain fitting software (LCModel version 6.2.2) to quantify brain metabolites.[17] An estimate of the variance associated (Cramer-Rao lower bounds) with time domain fitting provided by the software was used to determine reliability of the fitting and value of less than 15% was used to accept the fitting results for NAA, Cr, CHO and less than 25% is accepted for GLU.[18] Brain chemical concentrations are reported in mM uncorrected for T1, T2 relaxation times and contribution of gray and white matters in each voxel. In each voxel, we measured total choline (CHO), myo-inositol (MI), glutamate (GLU), creatine (CR), and n-acetyl aspartate (NAA). The same MRS head phantom measurements were acquired at the end of each examination. The quantitative results of the MRS phantom were consistent and reproducible throughout the 12-months.

Statistical analyses

The statistical analysis was conducted by first assessing the descriptive statistics for all MRS measures at all study time points by subject's HIV status (HIV infected vs. uninfected healthy controls). Two-sample t-test was used to assess the mean differences of MRS measures between healthy controls and HIV positive subjects. Paired t-test was used to examine the mean differences of MRS measures between baseline and each of the subsequent follow-up visits. The statistical tests were not corrected for multiple comparisons.

A mixed effect model was used to examine how the MRS measures change over time following initiation of treatment. Multivariate analyses were performed by adding age, gender, years of education and time-varying (CD4, plasma VL) as covariates in the mixed models. Pearson's correlation coefficients were used to examine the correlation between MRS and clinical measures. The level of significance was $p < 0.05$. Data were analyzed using SAS version 9.2 and SPSS version 17.0 (IBM Corporation, USA).

RESULTS

Group composition

The HIV-infected and uninfected participants were similar in sex and age with mean ages of 35 and 34 years, respectively. Among HIV-infected participants, baseline mean CD4 t-lymphocyte count was 233 cells/ mm^3 and the mean $\log_{10}$ plasma HIV RNA was 4.83 copies/ml prior to cART. After 6 and 12 months of treatment, the mean CD4 t-lymphocyte count increased to 371 and 412 cells/mm^3, respectively while the $\log_{10}$ plasma HIV RNA levels decreased to 1.84 and 1.70, respectively (Table 1). All but three cases were undetectable (lower level of detection 50 copies/ml) at 12 months ($\log_{10}$ plasma HIV RNA of 2.22, 2.45, and 2.10, reduced from baseline $\log_{10}$ plasma HIV RNA levels of 5.64, 5.34, and 4.93 respectively). At baseline, 27 of the 59 HIV-infected participants were diagnosed with HAND (46%), including five with HIV-associated Dementia (HAD), 8 with Mild Neurocognitive Disorder (MND) and 14 with Asymptomatic Neurocognitive Impairment (ANI). All but one of the 59 HIV-infected participants was started on an NNRTI-based regimen, with the latter starting a PI-based regimen. During the first year of treatment, 12 subjects switched to an alternative regimen owing to adverse drug reactions, resistance, pregnancy, or co-enrollment in another study.

Cross-sectional baseline brain metabolites before treatment

We observed group difference between HIV-infected and uninfected participants in the FWM with HIV-infected participants having a higher MI ($p=0.040$) and CHO ($p=0.002$) in the BG and PCG ($p=0.022$) relative to HIV-uninfected participants. We also noted lower NAA in the PCG (weakly significant with $p=0.051$) and higher NAA in the BG ($p=0.047$) in the HIV-infected group (see Table, Supplemental Digital Content 1).

Effects of cART on brain metabolites

Longitudinal brain metabolite changes from pre-cART through 6 months and 12 months after the initiation of cART treatment were measured (See Figure 1). We observed a pattern

of reduced inflammatory markers after 6 months of cART in the BG with statistical significance reached for decreased CR (p=0.001), CHO (p=0.001) and MI (p=0.012). After 12 months of treatment further reduction of CR (p=0.001), CHO (p=0.004) and MI (p=0.026) was observed in the BG compared to baseline. Additionally, CR was significantly decreased after 6 months of treatment in the FWM (p=0.014). There were no significant changes in NAA. Compared to uninfected controls, only CR (p=0.012) and GLU (p=0.012) were significantly lower among HIV-infected participants at 12 months. No other metabolite/voxel pairs differed at 12 months compared to controls.

Further analysis using a multivariate mixed effect model revealed changes in brain metabolites between baseline and 6 months after cART treatment (estimated coefficient) including decreased NAA (est. coef =−0.94, p=0.031) in the FWM and CR in the PCG (est. coef. =−0.73, p=0.026). We noted an increase in MI from the FGM between baseline and 6 months (est. coef. =0.39, p=0.022) and 12 months (est. coef. = 0.40, p=0.021). We also noted an increase in BG MI at 12 months compared to baseline (est. coef. =0.99, p=0.038) (see Table, Supplemental Digital Content 2).

Effects of clinical measures on brain metabolites

At baseline, there were direct correlations between plasma HIV RNA and MI in the BG (r=0.358, p=0.005) and the FGM (r=0.282, p=0.031). There were modest indirect correlations to the FWM NAA (r=−0.261, p=0.046). The pre-cART CD4 t-lymphocyte count indirectly correlated modestly with MI in the BG (r=−0.322, p=0.013) and FWM (r=−0.263, p=0.044). In separate analyses, a diagnosis of HAD at baseline (n=5) had a strong positive statistically significant correlation with BG Cr (r=0.972, p=0.006) and CHO (r=0.889, p=0.043). Further comparison among HIV-infected participants with normal cognition and those with HAND revealed increased MI in the FGM (p=0.014) at baseline. At 12 months participants who remained cognitively impaired showed increased CHO in the PCG (p=0.018) and decreased GLU in the FWM (p=0.027) and BG (p=0.013) (Table 2).

Correlation between changes in NPZ-global score and changes in brain metabolites revealed that decreased BG CHO from baseline to 12 months was correlated with improvement on the NPZ-global (r=−0.32, p=0.023). A decrease in MI also correlated with improved NPZ-global in the FWM (r=−0.43, p=0.002) and BG (r=−0.42, p=0.002) (See Table, Supplemental Digital Content 3). There were no significant correlations between changes in NPZ-global score and changes in brain metabolites from baseline to 6 months.

DISCUSSION

Several studies, including our recent studies, have demonstrated persistent and continued neuronal injury in treated HIV patients using proton MRS and MRI[12, 19–21]. Risk factors associated with neuronal injury and cognitive impairment includes plasma HIV DNA and pre-cART CD4 t-lymphocyte cell counts. There remain large gaps in our understanding of the underlying mechanism that contributes to the persistent injury despite cART.

This study identified brain chemical differences between pre-cART HIV-infected subjects and controls and between HIV-infected subjects with and without HAND post-cART. The

most robust baseline associations were with clinical parameters of plasma HIV RNA and CD4 t-lymphocyte cell counts at baseline. The elevated CHO noted at baseline between HIV-infected compared to uninfected subjects is consistent with expectations, supporting an inflammatory phenotype with untreated chronic HIV. In a preclinical study, elevated CHO/CR was observed in the frontal brain of SIV macaques at the time of peak viremia indicting early neuroinflammatory processes[22]. In human studies, elevated CHO/Cr has been documented in international settings[21, 23–25] and in the US populations[26–29]. However, in similar but smaller studies, Suwanwelaa et al observed no statistical difference of CHO/CR in asymptomatic HIV-infected participants,[30] while Winston et al. observed lower CHO/CR in HIV-infected participants prior to cART treatment compared to healthy volunteers[31]. One possible reason for this discrepancy is the assumption that CR is stable in HIV, an assumption that is not supported by our current and past data.

A pattern of improved CHO was observed across multiple voxels, and seemed to occur as early as 6 months, demonstrating more rapid recovery, which is consistent with findings from a recently reported multi-center study that identified normalization of CHO over two years.[32] At 12 months, we noted no evidence of continued elevation of CHO with levels that were similar to controls across all voxels.

Creatine, a combination of creatine and phosphocreatine measured with proton MRS, is a metabolite associated with cell energy metabolism. It is widely considered to be unchanged in numerous brain pathologies and is often used as an internal reference with metabolites, which are reported as a ratio to the presumed stable CR.[33–34] We observed a decrease in CR in multiple brain regions over time in these participants who initiated cART. Chang et. al. reported a similar finding in the frontal cortex of HIV-infected participants.[35] The level of CR in the brain is in equilibrium with phosphocreatine via the creatine kinase enzymes activity.[36] Our patients were treated with NNRTIs, which has been linked to reduced creatine kinase activity, raising concerns that they impact MRS CR; thus, it should not be considered to remain stable in this scenario.[37–38] Alternatively, it has been hypothesized that changes in total CR levels are associated with intense neuronal activity and failure of energy production.[39] High energy demands of infected monocytes during the early stage of HIV infection could persist in chronic HIV infection.[40]

Myoinositol is present mainly in glial cells and considered to be an important brain osmolyte.[41] It is only observable at a short echo time MRS, as used in our study. Elevated MI has been reported at various stages of HIV infection.[10, 42] In the present study, elevated MI in the FWM and slightly elevated MI in the BG at baseline reflected microglial activation during the early stage of HIV infection and tend to normalize after 12 months of treatment. This normalization suggests reduced inflammation and improved brain repair mechanisms after cART.

Unsettled issues remain around cognitive impairment in cART treated subjects, with increasing evidence of contributions from non-HIV-related comorbidity as well as HIV. In the current study, we demonstrated improved neuropsychological testing performance with cART and associations between HAND and MRS parameters. This provides further evidence of inflammatory pathogenesis to HAND. Among those who continued to have

impairment at 12 months, we continued to see elevated CHO at PCG, providing evidence that, at least among a subset of subjects, inflammation is incompletely resolved with cART.

Glutamatergic neurotransmission has been associated with the pathophysiology of cognitive dysfunction in several brain conditions including HAND.[43–46] Glutaminase is the enzyme that catalyzes the conversion of glutamine to glutamate and its activity has been shown to inhibit glutamate production in HIV.[47] Reduced GLU may reflect mitochondria damage[38, 48], enhanced synthesis of anti-oxidant[49–50] or glial injury[47, 51]. In the longitudinal component of our study, we observed lower GLU in FWM and BG among impaired compared to unimpaired subjects at 12 months. It is important to point out that several studies exist documenting the difficulty in sorting out GLU signal without contamination of the overlapped glutamine (GLN) signal at 1.5T[8, 52–53]. Using short echo time proton MRS, the unresolved GLU and GLN resonances are readily reported as GLX, a combination of GLU and GLN. Previous studies reported decreased GLX in FWM in cognitively impaired HIV-infected subjects[54] while increased GLX was observed in the BG in HIV participants who were on stable cART[55]. There are several factors that contribute to this discrepancy, which may include the scanner field strength (1.5T vs. 3T). Mohamed et al argued that changes in GLX might be due to dysfunction in the glutamate-glutamine cycle, therefore it is uncertain that increased GLX is a direct result of increase GLU or both GLU and GLN. Using a more rigorous MRS approach to measure the uncontaminated GLU signal at 3T, reduced GLU was also observed in the FWM of cognitively normal HIV-infected participants[8].

In the present study, the standard deviation for the GLU metabolite quantification using the LCModel analysis was > 20% (range 9–24%) in 8% of our results at 12 months. Therefore, we adopted criteria to include results in the final analysis of < 25%, which is higher than generally accepted (< 20%)%[18]. This practice is not unusual and has been used in several MRS studies[56–58]. We also noted that by not having follow up examinations in the uninfected participants, we might have missed some inherent variability in our measurement. However, there were no significant changes observed in MRS phantom measurements acquired at the end of each examination.

In summary, we identified evidence of CNS inflammation in HIV prior to cART and among those who fail to improve cognitively at 12 months. MRS inflammatory markers are linked to important clinical measures pre-cART including plasma HIV RNA, and neuropsychological testing improvement is associated with improvement in these MRS markers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

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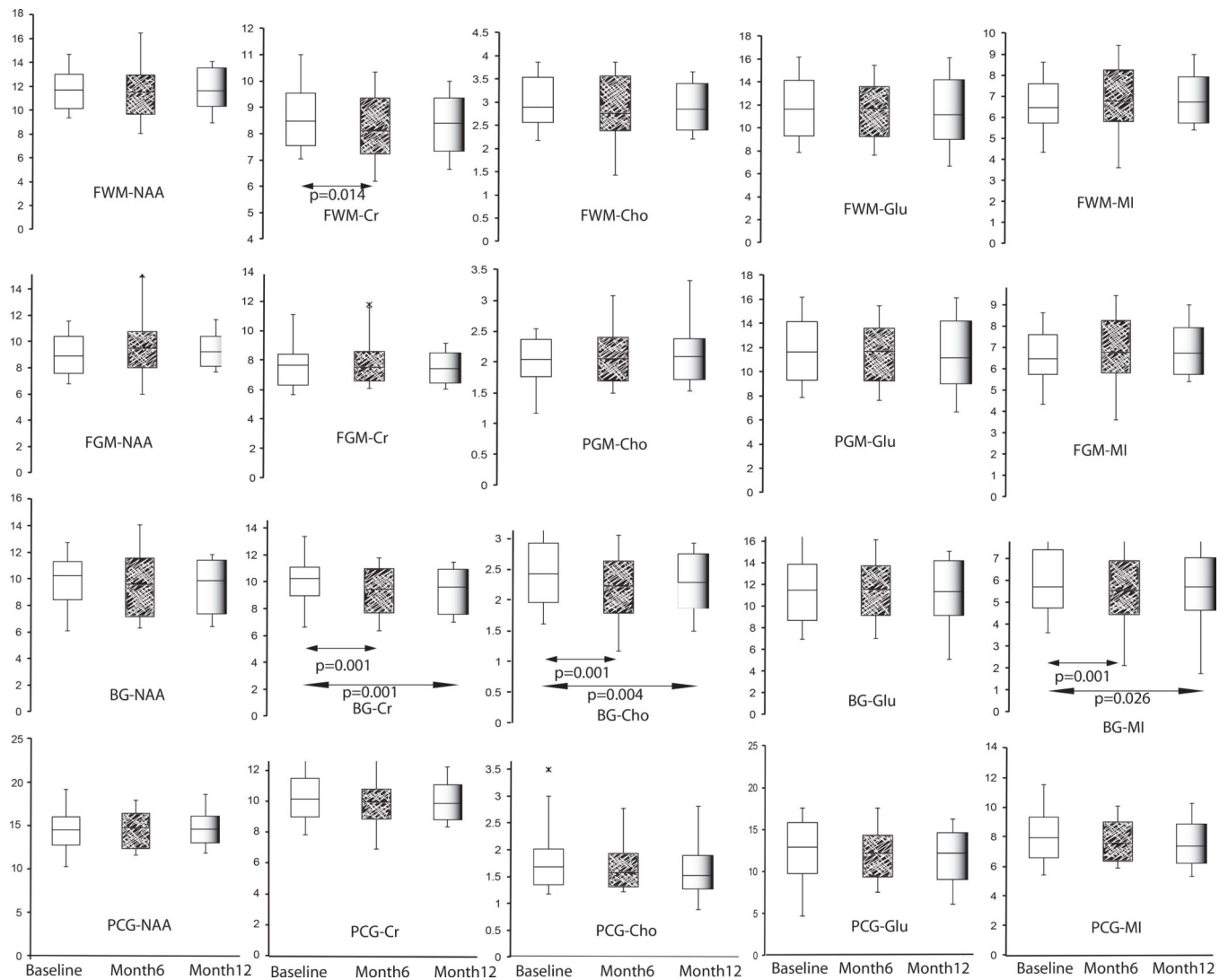**Figure 1.**
Longitudinal changes in MRS measures across the three time points (pre-cART, 6 months, and 12 months post cART) in HIV-infected subjects. A paired t-test is used with box plot with whiskers at 10th and 90th percentile.

Table 1

Participant demographics.

	HIV-infected	HIV-uninfected	*p*
N	59	28	
Mean (SD) age, years	35.0 (6.9)	34.3 (6.4)	0.47
Gender, M/F	25/34	15/13	0.22
Education, years	12(4.5)	14(5.2)	0.07
Mean/Median (SD) CD4 count cells/mm^3			
at enrollment	233		
at 6 months	371		
at 12 months	412		
Mean (SD) log$_{10}$ plasma HIV RNA			
at enrollment	4.83		
at 6 months	1.84		
at 12 months	1.70		

Table 2

MRS measures from the four brain locations by impairment status at baseline and 12 months.

	BASELINE			12 MONTHS		
	HIV+/HAND– (N=32)	HIV+/HAND+ (N=27)	p-value	HIV+/HAND– (N=41)	HIV+/HAND+ (N=9)	p-value
Choline						
FWM	2.91 (0.32)	3.04 (0.43)	ns	2.89 (0.38)	2.92 (0.31)	ns
FGM	2.03 (0.26)	2.05 (0.25)	ns	2.13 (0.31)	2.02 (0.37)	ns
BG	2.41 (0.33)	2.43 (0.36)	ns	2.27 (0.34)	2.30 (0.46)	ns
PCG	1.67 (0.43)	1.72 (0.25)	ns	1.54 (0.25)	1.80 (0.43)	p=0.018
N-acetyl aspartate						
FWM	11.91 (1.06)	11.60 (1.26)	ns	11.82 (1.31)	11.27 (1.15)	ns
FGM	9.15 (1.21)	8.90 (1.22)	ns	9.42 (0.83)	9.25 (1.01)	ns
BG	10.14 (1.19)	9.87 (1.38)	ns	9.63 (1.54)	9.04 (1.48)	ns
PCG	14.39 (1.45)	14.30 (1.67)	ns	14.65 (1.24)	14.86 (1.44)	ns
Myo-inositol						
FWM	8.91 (1.62)	8.90 (2.17)	ns	8.24 (1.21)	7.98 (0.89)	ns
FGM	6.43 (0.67)	6.97 (0.98)	p=0.014	6.85 (0.89)	6.84 (0.64)	ns
BG	5.92 (1.19)	6.38 (1.68)	ns	5.74 (1.17)	5.41 (0.83)	ns
PCG	7.71 (1.02)	8.18 (1.20)	ns	7.56 (1.09)	7.22 (1.17)	ns
Glutamate						
FWM	10.31 (1.53)	10.47 (2.02)	ns	10.55 (1.92)	8.96 (1.80)	p=0.027
FGM	11.75 (1.83)	11.32 (2.11)	ns	11.71 (2.00)	10.87 (2.03)	ns
BG	5.92 (1.19)	6.38 (1.68)	ns	11.82 (1.94)	9.88 (2.48)	p=0.013
PCG	7.71 (1.02)	8.18 (1.20)	ns	13.00 (1.97)	12.96 (1.20)	ns
Creatine						
FWM	8.43 (0.57)	8.61 (1.00)	ns	8.28 (0.84)	8.22 (0.75)	ns

	BASELINE			12 MONTHS		
	HIV+/HAND− (N=32)	HIV+/HAND+ (N=27)	p-value	HIV+/HAND− (N=41)	HIV+/HAND+ (N=9)	p-value
FGM	7.43 (0.98)	7.65 (0.92)	ns	7.56 (0.77)	7.19 (0.75)	ns
BG	10.26 (1.04)	10.05 (1.02)	ns	9.59 (1.11)	8.87 (1.34)	ns
PCG	10.03 (1.07)	10.45 (1.02)	ns	9.80 (0.92)	10.32 (0.54)	ns

FWM=frontal white matter; FGM=frontal grey matter; BG=basal ganglia; PCG=posterior cingulate gyrus Cr=creatine; Glu= glutamate, MI=myo-inositol; NAA=N-acetylaspartate, Cho=total choline