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Mitochondrial toxicity before and after combination antiretroviral therapy, a Magnetic Resonance Spectroscopy study

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ABSTRACT

The aim of this study was to quantify, via Magnetic Resonance Spectroscopy (MRS), the effect of combination antiretroviral therapy (cART) on brain metabolites and characterize any possible associations between changes in metabolites, age, blood biomarkers of neuronal damage, functional connectivity and cognitive performance. As cART has dramatically increased the life expectancy of HIV-infected (HIV +) individuals and unmasked an increase in HIV-associated neurocognitive disorders, it is still not clear whether cART neurotoxicity contributes to these disorders. We hypothesized a bimodal effect, with early cART treatment of HIV infection decreasing inflammation as measured by MRS metabolites and improving cognitive performance, and chronic exposure to cART contributing to persistence of cognitive impairment via its effect on mitochondrial function. Basal ganglia metabolites, functional connectivity, cognitive scores, as well as plasma levels of neurofilament light chain (NfL) and tau protein were measured before and after 12 weeks, 1 year and 2 years of cART in a cohort of 50 cART-naïve HIV + subjects and 72 age matched HIV- healthy controls. Glutamate (Glu) levels were lower in the cART naïve patients than in healthy controls and were inversely correlated with plasma levels of NfL. There were no other significant metabolite differences between HIV + and uninfected individuals. Treatment improved Glu levels in HIV+, however, no associations were found between Glu, functional connectivity and cognitive performance. Stable brain metabolites and plasma levels of NfL and Tau over two-years of follow-ups suggest there are no signs of cART neurotoxicity in this relatively young cohort of HIV + individuals.

1. Introduction

Combined antiretroviral therapy (cART) has dramatically altered the clinical course of HIV infection and increased the life expectancy of HIV-infected individuals (Bryant et al., 2015). Increased survival is associated with a rise in age-related comorbidities, substance use and psychiatric disorders, resulting in additional brain injury and possibly an increase in HIV-associated neurocognitive disorders (HAND) (Nabha et al., 2013; Saylor et al., 2016). There is concern that this trend may also be due to long-term effects of cART induced neurotoxicity (Marra et al., 2009; Schweinsburg et al., 2005). Animal studies suggest that treatment with nucleoside reverse transcriptase inhibitors (NRTI) is associated with CNS mitochondrial toxicity and may also affect mitochondrial endothelial function (Jiang et al., 2010; Venhoff et al., 2010).

Imaging studies have been used to study pathophysiology of HIV infection in the brain. Magnetic Resonance Spectroscopy (MRS) can non-invasively measure glial function and CNS inflammation and has been widely used to study HIV-associated CNS injury (Cohen et al., 2010; Harezlak et al., 2011) and HIV-associated cognitive impairment (Chang et al., 1999a, 1999b; Schifitto et al., 2007). N-acetyl aspartate (NAA), a marker of neuronal integrity, choline (Cho) and myo-inositol (mI), markers of inflammation, glutamate (Glu) and glutamine (Gln) have been shown to be abnormal in the basal ganglia (BG) of HIV + individuals (Harezlak et al., 2011). A decrease in NAA was a strong

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predictor of cognitive transition from normal to cognitive impairment and significant increases in markers of inflammation Cho and mI ratios to creatine (Cre) were observed in both HIV-positive neuro-asymptomatic subjects and subjects with AIDS Dementia Complex (Harezlak et al., 2011). Cognitive and metabolite abnormalities have been reported to persist after administration of antiretroviral drugs, indicating that inflammation and neuronal injury persist in chronic and stable HIV infection (Boban et al., 2017; Harezlak et al., 2011). MRS has been widely used to study healthy aging of the brain, consistently showing declining NAA and Glu concentrations and increasing Cho and Cre concentrations with older age in multiple brain regions (Chang et al., 2009; Haga et al., 2009; Kaiser et al., 2005; Marsman et al., 2013; Sailasuta et al., 2008). HIV and aging have been shown to independently impact brain health (Ances et al., 2010; Justice, 2010), but studies also suggest that risk of age-associated disease is higher in antiretroviral-treated HIV patients (Deeks, 2011) and that mitochondrial toxicity and oxidative stress contribute to premature aging in this population (Caron-Debarle et al., 2010). Research indicates that Glu mediated excitotoxicity may contribute to HIV brain injury and that brain Glu on MRS may provide an early surrogate marker for monitoring disease severity and treatment effects (Ernst et al., 2010).

Neurofilament light chain (NfL) is a promising new biomarker for neurodegeneration which can be measured both in the brain cerebrospinal fluid (CSF) and in the peripheral blood. Increased levels of NfL, and total tau protein (Tau) concentrations in cerebrospinal fluid (CSF) have been associated with neurodegeneration (Abdulle et al., 2007; Peterson et al., 2014) and cognitive impairment in HIV infected patients (Steinbrink et al., 2013). A decrease or normalization of NfL concentration in CSF after cART initiation strongly suggests that the elevated NfL pre-treatment concentration is due to the neurodegenerative process caused by HIV, rather than other confounding neurologic injury (Mellgren et al., 2007), and therefore can be used as a biomarker of brain injury due to HIV infection. NfL is a marker of neuronal injury which would be expected to be inversely correlated with levels of NAA. Neuroinflammation as measured by increased mI and Cho could be positively correlated with NfL if neuronal injury had also occurred. However, the sensitivity of NfL vs. MRS metabolites is yet to be established. To our knowledge only one study has investigated this and found no association between neurometabolite concentrations and neuronal damage markers in blood or CSF in perinatally HIV-infected children (Van Dalen et al., 2016). While MRS measurements of metabolites is non-invasive, sampling of CSF to measure NfL biomarkers is limited by the invasiveness of the lumbar puncture procedure. Collection of blood is both inexpensive and noninvasive, and therefore preferable to both lumbar puncture and expensive MRI exams. Research in HIV has demonstrated that blood and CSF NfL levels were highly correlated and detectable in both severe and subclinical neuronal injury in HIV as well as in HIV negative controls, even though the level of the biomarker in plasma is 50-fold lower than in CSF (Gisslén et al., 2016).

Resting state functional connectivity (FC) is a measure of temporal correlation in spontaneous blood oxygen level dependent (BOLD) fluctuations and has been used to characterize large-scale neural networks (Damoiseaux et al., 2006). In HIV infection, studies of the most well-characterized brain network, the default-mode network (DMN), have shown altered within network connectivity, with lower connectivity in the HIV + population compared to healthy controls, that improved after cART treatment to a level comparable to that of healthy controls (Thomas et al., 2013; Zhuang et al., 2017). It has also been reported that effects of HIV and aging were additive as revealed by intranetwork FC measures of the DMN. (Thomas et al., 2013). Resting state FC is also sensitive to HIV pathology within subcortical structures as demonstrated by altered cortico-striatal FC, including that between the striatum and DMN (Ortega et al., 2015). This is consistent with HIV neuroimaging, pathological, virological and metabolic studies that indicate that the basal ganglia are a major target of HIV infection (Berger and Arendt, 2000). Previous work in healthy subjects found that resting-state cortical-subcortical FC is positively correlated with glutamate concentration in the medial prefrontal cortex(Duncan et al., 2013) and that subcortical Glu concentration mediates local connectivity during human development (Ghisleni et al., 2015). To our knowledge no studies have examined the relationship between basal ganglia Glu and DMN FC in HIV infection, nor if there is an effect of cART treatment.

The primary aim of this study was to quantify, via MRS, the effect of early cART exposure on brain metabolites in the BG. Secondarily, we also sought to determine any possible associations between changes in brain metabolites and age, blood biomarkers of neuronal damage, and cognitive performance, as well as determine if Glu concentration in BG, known to be altered in HIV, had any mediating effects on the FC of BG and DMN.

2. Methods

The study was approved by the institutional Research Subjects Review Board and all subjects signed a written consent prior to undergoing study procedures. A total of 122 subjects, 50 recently diagnosed and treatment-naïve HIV+ (46 male, mean age 35.54 ± 12.5) and 72 age matched HIV- healthy controls (38 male, mean age 38.89 ± 12) were enrolled. Demographic characteristics of subjects included in these analyses are presented in Table 1.

All participants underwent a comprehensive clinical, laboratory (chemistry, hematology, and urine analysis), neurocognitive and neuroimaging evaluation. HIV + individuals were assessed at baseline (BSL-before starting cART), 12-weeks (W12), 12 months (Y1) and 24 months (Y2), while the HIV- were assessed at BSL, Y1 and Y2. All HIV + participants were antiretroviral treatment-naïve prior to enrollment. They met the following laboratory parameters within 30 days of baseline evaluation: hemoglobin $\geq$ 9.0 g/dL, serum creatinine $\leq$ 2 × ULN, AST (SGOT), ALT (SGPT), and alkaline phosphatase $\leq$ 2 × upper limit of normal. Participants with severe premorbid or comorbid psychiatric disorders such as diagnoses of schizophrenia, bipolar disorder and active depression were excluded. Subjects with mild or stable depression including those on stable antidepressant therapy were eligible. Additional exclusion criteria were stroke, head trauma resulting in loss of consciousness >30 min, multiple sclerosis, brain infections (except for HIV-1), and any space-occupying brain lesions requiring acute or chronic therapy. Dementia, as established by HAND definition (Antinori

Table 1
Demographic characteristics. Duration of disease was from date of diagnosis to study enrollment. SE-standard Error; VL-Viral Load; InI-Integrase Inhibitor; NNRTI- Non-Nucleoside Reverse Transcriptase Inhibitor; other- complex combinations.

		HIV+ (n = 50)	HIV- (n = 72)
Age, Mean (SE)		35.54 (1.77)	38.89 (1.41)
Sex, n (%)			
	Female	6 (12%)	34 (47.2%)
	Male	44 (88%)	38 (52.8%)
Education, n (%)			
	Less than or equal to 12 Years	16 (32%)	8 (11%)
	More than 12 Years	34 (68%)	64 (89%)
HIV duration at baseline years, Mean (SE)		1.69 (0.71)	–
CD4 at baseline cells/uL, Mean (SE)		510.62 (38.11)	–
VL at baseline copies/mL, Mean (SE)		72171.04 (18201.6)	–
NfL (pg/mL) Mean (SE)		12.86 (2.7) (n = 48)	7.32 (0.56) (n = 15)
Tau (pg/mL) Mean (SE)		2.47 (0.16) (n = 48)	2.32 (0.18) (n = 15)
Total Cognitive Score, Mean (SE)		−2.01 (0.56)	0.07 (0.49)
Drug classification	InI, n (%)	36 (72%)	–
	NNRTI, n (%)	7 (14%)	–
	other, n (%)	7 (6%)	–

et al., 2007), was exclusionary.

Subjects meeting criteria for HIV-associated mild neurocognitive disorder (MND) or HIV-associated asymptomatic neurocognitive impairment (ANI) were included. Active alcohol and drug abuse (urine toxicology was done at each visit) within 6 months of study entry and conditions such as claustrophobia or metallic implant that prevented MRI scanning were exclusionary.

Neuropsychological tests: The neurocognitive evaluation included tests of executive function (Trailmaking Test Part B, Stroop Interference Task), speed of information processing (Symbol Digit Modalities Test and Stroop Color Naming), attention and working memory (CalCAP), learning (Rey Auditory Verbal Learning Test (AVLT Trials 1–5), Rey Complex Figure Test Immediate Recall), memory (RAVLT Delayed Recall, Rey Complex Figure Test Delayed Recall), and motor (Grooved Pegboard, the left and right hands). An estimate of premorbid intellectual functioning ability was obtained via WRAT-4 Reading. The total summary cognitive score was the primary cognitive outcome and was created as the summation of the Z-scores of the seven cognitive domains measured (executive function, speed of information processing, attention and working memory, learning, memory, verbal fluency and motor). HAND diagnoses were determined for each participant according to the Frascati criteria (Antinori et al., 2007).

MRI data acquisition and analysis: MRI was performed on a 3 T Siemens Trio MRI scanner equipped with a 32-channel head coil. A T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE, repetition time (TR)/inversion time (TI)/echo time (TE) = 2530/1100/3.44 ms, voxel size = $1 \times 1 \times 1$ mm^3, flip angle = 78°, bandwidth = 190 Hz/pixel) was acquired. A single $15 \times 15 \times 15$ mm^3 voxel proton spectrum was acquired using a PRESS sequence from the BG (deep GM, Fig. 1), TR/TE = 2000/35 ms, 128 averages. Manual 1st and 2nd order shimming was performed to improve magnetic field homogeneity, critical to obtain narrow linewidths for accurate metabolite quantification and efficient water suppression (Spielman et al., 1998; Wilson et al., 2019). The voxel was placed either in the left or right BG, depending on which side we achieved the best shimming, i.e. the narrowest water peak linewidth.

Metabolite concentrations were determined with LCModel software (version 6.3) (Provencher, 1993, 2001), which analyzes the spectra as a linear combination of a set of model spectra of metabolite solutions in vitro. For all spectroscopic data, the reference basis set for 3-Tesla PRESS sequence with TE = 35 ms was used. Metabolite concentrations with uncertainties (Cramer-Rao lower bounds) larger than 20%, as given by LCModel, were not included in the statistical analysis. An unsuppressed water reference signal was combined with water-suppressed data to estimate absolute metabolite concentrations, in institutional units.

Resting-state fMRI scans were acquired using a gradient echo-planar imaging (EPI) sequence (TR/TE = 2000/30 ms, voxel size = $4 \times 4 \times 4$ mm^3, flip angle = 90°, 30 axial slices, 150 time points). During the entire 5-min resting-state fMRI scanning, participants were instructed to keep their eyes closed and avoid falling asleep. Standard pre-processing steps were performed using FSL (FMRIB's Software Library) FEAT tool (Woolrich et al., 2001), and included slice timing correction, head motion correction, co-registration to T1 image, normalization, spatial smoothing with Gaussian kernel (full-width half-maximum = 4 mm), detrending, and band-pass filtering (0.01 Hz to 0.08 Hz). Nuisance covariates including head motion parameters and white matter and CSF signals were regressed out. After pre-processing, the group independent component analysis (ICA) of fMRI Toolbox (GIFT, http://mialab.mrn.org/software/gift/index.html) was used to produce t-score maps reflecting functional maps of 30 components. Maps representing the BG and DMN were selected and masks were generated using voxels with t-statistic corresponding to p-value < 0.05. The masks were then applied to the pre-processed fMRI images, and the FC was calculated as the Pearson correlation within and between networks.

Blood biomarkers: Blood samples were collected from all HIV + subjects and a small subsample of healthy controls at baseline and year 2, to use as reference. Plasma neurofilament light chain (NfL), and total tau protein (Tau) levels were obtained using the Simoa platform (Quanterix Corporation, Billerica MA, USA). Only 63 subjects (48 HIV + and 15 HIV-) with samples from at least two time points were included in this analysis, to evaluate changes in these markers with time and treatment.

Statistical analysis: Comparisons between two independent groups (e.g., HIV + versus HIV- subjects at baseline) were conducted by either two-group Welch's *t*-test (for continuous variables such as the concentrations of MRS metabolites) or Fisher exact test (for categorical variables such as sex and race). Paired t-tests were used to compare the levels of continuous variables in HIV + subjects between BSL and W12.

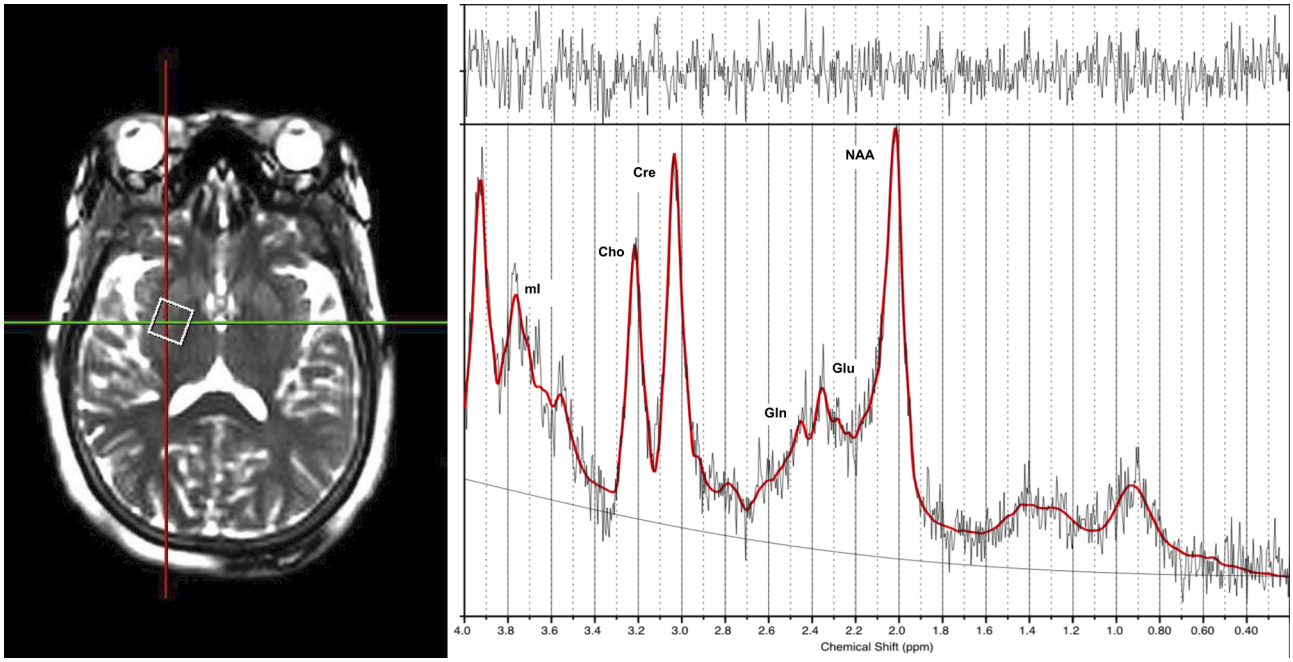

Fig. 1. MRS voxel placement and a representative LCModel spectrum for an HIV + subject at baseline.

In these analyses, parameters were estimated by the restricted maximum likelihood (REML) criterion, and the statistical significance was assessed by the adjusted ANOVA F-test provided by the R package lmerTest (Kuznetsova et al., 2017). For inferential problems that involved multiple hypotheses, Benjamini–Hochberg multiple testing procedure (Benjamini and Hochberg, 1995) was used to control the false discovery rate (FDR) at < 0.05 level. All statistical analyses were performed in R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

Effects of HIV status and age on MRS metabolites. Linear mixed effects regression (LMER) models were used to quantify the association between selected covariates and continuous response variables, with per-subject random intercepts to account for serial correlation between multiple time points. Empirical evidence showed that the cART treatment effect was most prominent in the first 12 weeks; its long-term effect was subtler and, in some cases, different from its short-term effect. Therefore, we performed two separate LMER analyses to study the longitudinal associations with MRS metabolite concentrations.

1. The Short-Term Model (**STM**) refers to the first arm of our hypothesized bimodal effect, i.e the acute exposure to cART. It was applied to data collected at BSL and W12 for the HIV + group. Covariates included were short-term cART treatment (visit), age, and the interaction between age and visit to account for possibly different patterns of cART treatment effects between young and older subjects.
2. The Long-Term Model (**LTM**) refers to the second arm of our hypothesized bimodal effect, i.e. the chronic exposure to cART. was applied to data collected from HIV- subjects at BSL and HIV + subjects at W12 (used as the new baseline in LTM), Y1 and Y2 data collected from both cohorts. Covariates included were HIV status, visit, age, the interaction between age and visit, and the interaction between HIV status and visit.

Association of cognitive scores and MRS metabolites. We adapted the STM and LTM to determine the association between summary cognitive scores and MRS metabolites. Specifically, for each metabolite, we conducted STM and LTM which included the selected metabolite, HIV status, visit, age, and the interaction term between visit and age as covariates. Random intercepts were included to account for between-subject variation and serial correlation. Summary cognitive scores were considered as the response variable in these models. These models were applied to both HIV + and HIV-.

Blood biomarker effects. In the subset of 63 subjects with blood biomarkers, we examined the relationship between these biomarkers, brain metabolites and cognitive scores.

Association of biomarkers with brain metabolites: We performed Spearman Correlation Test between brain metabolites and average concentrations (pg/ml) of NfL and Tau at baseline. We applied the STM and LTM to study the associations between NfL/Tau average concentrations (pg/ml) levels and metabolite concentrations in HIV + subjects. Covariates included were NfL or Tau, visit and age.

Association of biomarkers with cognitive scores: We applied the STM and LTM to study the joint associations between NfL/Tau average concentrations (pg/ml) levels and cognitive scores for n = 48 HIV + subjects. Covariates included were NfL or Tau, visit, age, and the interaction between visit and age (for LTMs only).

Association of FC within and between BG and DMN networks with Glu concentration: We used the STM and LTM to determine the association between FC as the outcome variable, and covariates included Glu concentration, HIV status, visit, age and the interaction term between cohort and visit. A linear regression of FC measures as outcome and Glu, HIV status, and age as covariates was conducted at baseline only to determine if there were differences between HIV + and HIV-.

3. Results

3.1. Participant characteristics

There was no statistically significant age difference between HIV- and HIV + subjects (p = 0.1427), but we included age as a covariate in all multivariate regression analyses to control for remaining confounding effects. There was attrition in both HIV + and HIV- groups, the number of remaining subjects at each time point is presented in Table 2 along with their clinical characteristics and cognitive scores.

3.2. Effects of HIV status and cART treatment on MRS metabolites

HIV + vs HIV- at BSL. Three subjects (two HIV + and one HIV-) with noisy MRS spectra and metabolite concentrations having uncertainties (Cramer-Rao lower bounds) larger than 20%, were removed. Comparisons between the remaining subjects (n = 48 for HIV + and n = 71 for HIV-) showed that most metabolite concentrations and ratios were lower in HIV + than in HIV-, but the difference was not statistically significant. The difference between HIV + and HIV- subjects in Glu absolute concentration was significant (HIV+=6.71, HIV-=7.34, p = 0.025) in univariate analyses (Table 3, Fig. 2).

Short Term effects of cART: In paired comparison between BSL and W12 for HIV + subjects (n = 36 subjects with measures at both BSL and W12), we found no significant differences in metabolite concentration levels (Table 4). Of note, even though not statistically significant there was an increase in average Glu concentration (BSL Glu = 6.546, W12 Glu = 7.156, p = 0.162), with W12 value closer to that of healthy controls (HIV- BSL Glu = 7.34, p = 0.351) (Fig. 2).

Long Term effects of cART: There were no significant longitudinal differences in any of the metabolite concentrations as determined by the marginal paired t-tests between W12 and Y1, as well as Y1 and Y2. The Long-Term Model (LTM) also revealed no significant longitudinal changes in any of the metabolites (see Fig. 2 for Glu levels at each visit). The lack of significant visit effect in the LTM suggests that levels of metabolites in the HIV + subjects were stabilized after 12 weeks of cART treatment.

Table 2
Table 2. Demographic, clinical and cognitive characteristics of subjects at follow up visits. SE-standard Error; VL-Viral Load; VL undetectable- number and percentage of HIV + subjects who had VL at<20 copies/mL.

	HIV + W12 (n = 38)	HIV +Y1 (n = 31)	HIV + Y2 (n = 23)	HIV- Y1 (n = 58)	HIV- Y2 (n = 51)
Age, Mean (SE)	36.05 (2.17)	35.58 (2.36)	35.65 (2.78)	39.86 (1.53)	40.92 (1.59)
Sex, n (%)					
Female	3 (7.9%)	4 (12.9%)	3 (13.0%)	27 (46.6%)	22 (43.1%)
Male	35 (92.1%)	27 (87.1%)	20 (87.0%)	31 (53.4%)	29 (56.9%)
CD4 cells/uL, Mean (SE)	596.42 (47.24)	691.29 (62.59)	728.43 (64.39)	–	–
VL copies/mL, Mean (SE)	432.49 (395.76)	1162.10 (1144.17)	30.30 (11.39)	–	–
VL undetectable, n (%)	24 (63.2%)	25 (80.6%)	16 (69.6%)	–	–
NfL (pg/mL), Mean (SE)	9.9 (1.59)	9.48 (1.83)	10.6 (2.3)	–	7.51 (0.59)
Tau (pg/Ml), Mean (SE)	2.33 (0.27)	1.95 (0.12)	2.24 (0.17)	–	2.04 (0.24)
Total Cognitive Score, Mean (SE)	−0.46 (0.60)	−0.73 (0.70)	−1.13 (0.98)	1.84 (0.61)	2.19 (0.70)

Table 3
Metabolite average concentrations (institutional units) and ratios to Cre for HIV+ (n = 48) and HIV- (n = 71) subjects at baseline, uncorrected p-value Metabolite concentration values were determined as ratio to the water signal in institutional units (i. u.), not molar concentrations.

MRS	HIV+	HIV-	p-value
NAA	5.83	6.09	0.311
Glu	6.71	7.34	**0.025**
Gln	6.76	6.52	0.507
GLX (Glu + Gln)	12.85	12.92	0.865
Cho	1.71	1.75	0.487
mI	3.71	3.83	0.555
Cre	6.55	6.83	0.067
NAA /Cre	0.88	0.90	0.634
Glu/Cre	1.02	1.08	0.244
Gln/Cre	1.01	0.95	0.330
GLX/Cre	1.94	1.89	0.367
Cho/Cre	0.27	0.25	0.183
mI/Cre	0.58	0.57	0.974

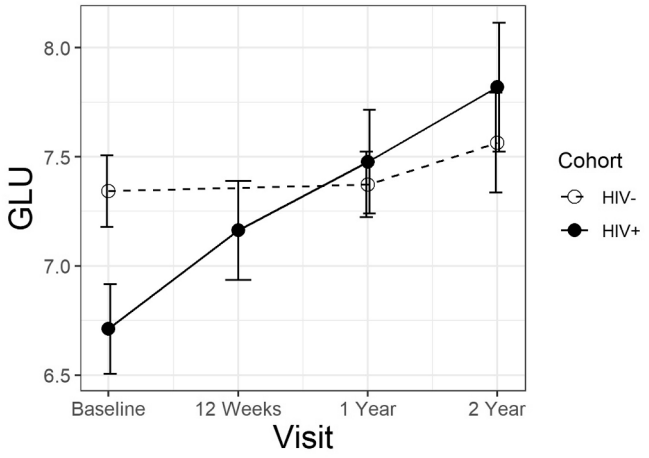

Fig. 2. Glutamine (GLU) levels at baseline and follow up visits for HIV + and HIV- subjects; number of subjects was different at each follow up visit (see Table 2). There was a significant difference between HIV + and HIV- at baseline (p = 0.025).

Table 4
Association between overall cognitive z-score and NfL or Tau average concentrations (pg/ml), for HIV + subjects (n = 48), STM: baseline and W12 and LTM: W12, Y1 and Y2.

MRS	BSL	W12	p-value
NAA	5.92	5.81	0.746
Glu	**6.55**	**7.16**	0.162
Gln	6.75	6.60	0.716
Cho	1.72	1.71	0.738
mI	3.66	3.63	0.922
Cre	6.66	6.79	0.425
NAA/Cre	0.88	0.85	0.553
Glu/Cre	0.99	1.03	0.737
Gln//Cre	1	0.97	0.913
Cho/Cre	0.26	0.25	0.309
mI/Cre	0.56	0.52	0.542

3.3. Association of cognitive scores and MRS metabolites

We found no significant relationships between brain metabolites and cognitive scores, neither short nor long term. In STM analyses we found that HIV + subjects performed significantly worse in cognitive tests than the controls at baseline (total cognitive score, β = -2.36, p = 0.018), and improved after 12 weeks of cART (total cognitive score, β = 1.64, p = 0.048). We did not find any statistically significant changes of total cognitive scores over the Y1 and Y2 follow-ups in either HIV + or HIV- groups. Despite of lack of statistical significance, it is interesting to point out that HIV + subjects showed mild declines of total cognitive scores while HIV- subjects showed mild improvements.

3.4. Blood biomarker effects in the HIV + cohort

Association of biomarkers with brain metabolites: By applying Spearman's correlation test to HIV + at BSL, we identified a significant negative association between NfL average concentration and Glu ($\rho = -0.318$, p = 0.049). No significant relationship between MRS and Tau was detected. In the STM and LTM models looking at associations between NfL/Tau average concentrations (pg/ml) levels and metabolite concentrations we found a significant negative association between NfL and Glu in the STM ($\rho = -0.041$, p = 0.023) and no relationship in the LTM.

Association of biomarkers with cognitive scores: NfL was found to be significantly associated with decreased cognitive scores in LTM (ρ = -0.08, p = 0.043). The observed association between NfL and cognitive scores was also negative in STM but not statistically significant (ρ = -0.02, p = 0.335).On the other hand, we did not identify any significant association between Tau concentration levels and cognitive scores. (Table 4).

Association of FC within and between BG and DMN networks with Glu concentration: At baseline there were significant associations of FC in BG with HIV status (lower in HIV+, p = 0.0015) and age (lower FC in older subjects, p = 0.03). There were no significant relationships between Glu concentration and FC within or between the BG and DMN networks, either on the short or long term. In the LTM, FC within the BG network decreased significantly between visits (p = 0.0002) and with age (p < 0.0005), and was lower for HIV+ (trend, p = 0.06) independent of Glu concentration. The FC within DMN also decreased significantly with age (p = 0.03), while the FC between the two networks increased between visits (p = 0.01). There were no significant changes in FC association with any of the covariates in the HIV + between BSL and W12.

4. Discussion

In this study we hypothesized a bimodal effect of the HIV virus and treatment neurotoxicity. We expected that early cART treatment of HIV infection would decrease inflammation and improve cognitive performance. However, chronic exposure to cART was expected to contribute to persistence of cognitive impairment via its effect on mitochondrial function. We found evidence supporting the first arm of our hypothesized model, where acute 12-week exposure to cART decreased inflammation and improved cognitive scores. However, the chronic exposure to cART did not appear to significantly affect inflammation nor cognition, therefore suggesting there was no significant cART neurotoxicity. These results suggest cART naïve patients with relatively preserved immune function have mild cognitive impairment and minimal brain metabolite abnormalities. These findings are in line with previous findings of metabolite abnormalities (Gongvatana et al., 2013) if we take into account that our HIV + group had a relative high CD4 count, was relatively young and included subjects with shorter durations of disease before they started treatment.

MRS provides a non-invasive assessment of brain metabolites reflecting neuronal and glia function. In our study most metabolite concentrations and metabolite ratios were lower in HIV + than in HIV- at baseline, but only Glu was significantly decreased in the basal ganglia of HIV + when compared to healthy controls. Findings of decreased levels of NAA in chronically HIV-infected subjects, but not in those with early infection were reported earlier (Lentz et al., 2009). It was also suggested that abnormalities in NAA reflect the subclinical brain impact of initial disease severity (Chu et al., 2018). Therefore, we suggest the relatively normal levels of NAA in our HIV + cohort at baseline are due

to the characteristics of our cohort, with short disease duration, relatively preserved immune function and mild cognitive impairment. However, even when the disease duration is short, the significantly lower Glu levels in basal ganglia in our HIV + cohort compared to healthy controls, followed by improvement on a 12-week cART regimen, suggest that Glu abnormalities are a very early sign of dysfunction in HIV, and that brain Glu as detected with MRS may constitute an early marker for monitoring disease severity and treatment effects. Glu is an amino acid and neurotransmitter with important roles in normal cell function and neurotransmission, but excessive concentrations of extracellular Glu may be neurotoxic and induce cell death (Kaul et al., 2001). Reduced Glu has been observed in HIV and has been suggested to reflect mitochondrial damage, enhanced synthesis of anti-oxidant or glial injury (Ernst et al., 2010; Sailasuta et al., 2016). Normalization of Glu levels after 12 weeks of cART may reflect reduced inflammation and improved brain repair mechanisms (Sailasuta et al., 2012). It has also been suggested that use of antiretroviral treatments may contribute to mitochondrial toxicity (Ernst et al., 2010; Kaul et al., 2001) through disruption of astrocyte functions (Vivithanaporn et al., 2016). One of the critical functions of astrocytes is to ensure the extracellular glutamate levels are maintained below excitotoxic levels to neurons (Vivithanaporn et al., 2016). Our findings of no changes in metabolite concentration while on chronic cART treatment for one or two years suggest that levels of metabolites in the HIV + subjects stabilized after 12 weeks of cART treatment and there was no significant cART neurotoxicity. Therefore, Glu neurotransmission may be an important target for early interventions to reduce neurotoxicity and the later incidence of neurocognitive impairment among HIV-positive patients (Vazquez-Santiago et al., 2014).

Cognitive impairment is a well-known complication of HIV infection (Antinori et al., 2007; Heaton et al., 2010). It is thought to be due to chronic neuro-inflammation associated with the infection. Therefore, the use of cART is anticipated to minimize inflammation, and thus cognitive impairment. Even in our relatively young cohort with short disease duration, we found that HIV + subjects performed significantly worse in cognitive tests than the controls at baseline and improved after 12 weeks of cART (Zhuang et al., 2020). However, previous studies reported that cognitive impairment persists despite optimal virological control (Saylor et al., 2016). Therefore, other contributing factors may be at play in the pathomechanism of cognitive impairment, such as long-term effects of cART induced neurotoxicity (Marra et al., 2009; Schweinsburg et al., 2005). A decrease in NAA was a strong predictor of cognitive transition from normal to cognitive impairment (Harezlak et al., 2011), but in our study the levels of NAA in HIV + were not significantly different than those of healthy controls, nor did they change with treatment. Minimal brain metabolite alterations and mild cognitive impairment of our cohort could also explain the lack of correlation between cognitive performance and metabolite levels described previously (Ernst et al., 2010). Short-term (12 weeks) cART treatment was associated with improvement in cognitive performance and normalization of Glu levels, with no deterioration over the two-year follow-up.

Interestingly, NfL plasma levels were negatively associated with Glu concentration and with cognitive performance. This finding is different than that of a previous study investigating neurometabolite concentrations and neuronal damage markers in perinatally HIV-infected children (Van Dalen et al., 2016), which found no association between metabolites and NfL. Increased levels of neurofilament light chain (NfL), and total tau protein (Tau) concentrations in cerebrospinal fluid (CSF) have been associated with neurodegeneration (Abdulle et al., 2007) and cognitive impairment in HIV infected patients (Steinbrink et al., 2013). We found a connection of one of these neurodegenerative markers to Glu levels in the BG of HIV + individuals before cART treatment. A decrease or normalization of NfL concentration after cART initiation strongly suggests that the elevated NfL pre-treatment concentration is due to the neurodegenerative process caused by HIV rather than other confounding neurologic injury (Mellgren et al., 2007), and therefore can be used as a biomarker for brain injury in HIV infection. Both NfL and Tau were negatively correlated with the cognitive score, but only the NfL association was significant, unlike Steinbrink et al., 2013 who found increased levels of both NfL and Tau concentrations to be associated with cognitive impairment in HIV infected patients. There are two main differences between their study and ours: their HIV + patients had a longer disease duration (time since seroconversion 8 ± 7 years) compared to ours (1.69 ± 0.71 years) and they measured the two markers in CSF while we did in plasma. It is known that, although they are highly correlated the levels of markers in CSF are about 50-fold higher than in plasma (Gisslén et al., 2016).

Although previous studies have shown that disease and aging independently impact brain health in HIV (Ances et al., 2010; Justice, 2010) or that risk of age-associated disease is higher in antiretroviral-treated HIV patients (Deeks, 2011), we have not found this in our study. We only observed normal age-related changes in both HIV + and HIV- groups. This may suggest that early detection and treatment initiation are beneficial in reversing any aging pattern associated with HIV infection (Boban et al., 2018). It is also possible that a longer observation may be necessary to further assess a putative cART neurotoxic effect.

At baseline, we found a significantly lower FC within the basal ganglia network of the HIV + group when compared to the HIV- group and a significant effect of age. Previous research showed lower connectivity in the default mode, control, and salience networks in the HIV + population compared to healthy controls (Thomas et al., 2013). We have not found any significant differences in FC within DMN in any of our comparisons. Our findings appear to be in line with a study by Wang et al. (2011), investigating rs-fMRI networks in HIV-infected patients within their first year of HIV infection. In this work a number of networks including DMN but not basal ganglia were examined and impaired FC was found only in the lateral occipital cortex network. Their findings along with ours may suggest that early in the disease process, FC is only altered in specific local networks. It is possible that early FC alterations in BG may facilitate later alterations in the DMN mediated by cortico-striatal connections. Previous research has reported altered cortico-striatal FC, including that between the striatum and DMN in either cART naïve or treated HIV patients with disease duration longer than 2 years (Ortega et al., 2015). After our patients became stable on cART treatment, the only effect we found long term was a significant decrease of FC within the BG with age independent of HIV status. This is in line with recent findings in a large sample of healthy older adults, of significant negative correlation between age and FC in basal ganglia structures (Griffanti et al., 2018). There were no significant relationships between Glu concentration and FC within or between the BG and DMN networks similar to the reported effects of Glu on either local or cortical-subcortical FC connectivity in healthy controls (Duncan et al., 2013; Ghisleni et al., 2015). This suggests that the relationship between Glu concentration and functional connectivity is not linear and may behave differently in the presence of pathology.

One of this study's limitations was the small sample size both due to enrollment restrictions and attrition. The number of HIV + subjects decreased by 15% at 12 weeks, and approximately 25% at the subsequent Y1 and Y2 visits. However, our unique study design allowed us to compare treatment naïve HIV + individuals to healthy controls to assess the effect of the virus and separate it from the short- and long-term effects of cART treatment, by evaluating the HIV infected individuals at 12 weeks and 1 and 2 years of treatment. In addition, the number of females in the HIV + group was much smaller than that of males, reflecting the higher rate of HIV-infection for males than for females. However, we did not consider this to have a significant effect on our results, as we found no sex related differences when we compared the more balanced male and female healthy control groups. Also, our intention to enroll treatment naïve individuals resulted in a relatively young population, which limited the evaluation of the differential effect of cART in older vs. younger HIV infected individuals.

5. Conclusion

Based on previous research we hypothesized a bimodal effect of cART treatment on HIV infection and cognition. Our results support part of the bimodal effect, i.e. early treatment decreases inflammation as measured by MRS metabolites and improves cognitive performance. However, in the population investigated, there is no evidence of mitochondrial dysfunction or cART neurotoxicity, as measured by MRS metabolites, plasma levels of NfL and Tau after two years of follow-up. This could signify that early diagnosis and cART treatment may mitigate neuroinflammation and thus long-term HIV-associated brain injury.

CRediT authorship contribution statement

Madalina E. Tivarus: Conceptualization, Methodology, Supervision, Writing - original draft. **Yuchuan Zhuang:** Data curation, Investigation. **Lu Wang:** Data curation. **Kyle D. Murray:** Data curation, Investigation, Writing - review & editing. **Arun Venkataraman:** Data curation. **Miriam T. Weber:** Methodology, Writing - review & editing. **Jianhui Zhong:** Conceptualization, Supervision, Writing - review & editing. **Xing Qiu:** Methodology, Supervision, Writing - review & editing. **Giovanni Schifitto:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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