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Systemic Mitochondrial Oxidative Phosphorylation Protein Levels Correlate with Neuroimaging Measures in Chronically HIV-Infected Individuals

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Abstract

Few studies have examined systemic mitochondrial function in conjunction with brain imaging in human immunodeficiency virus (HIV) disease. Oxidative phosphorylation enzyme protein levels of peripheral blood mononuclear cells were measured in association with neuroimaging indices in 28 HIV+ individuals. T1-weighted magnetic resonance imaging yielded volumes of seven brain regions of interest; diffusion tensor imaging determined fractional anisotropy (FA) and mean diffusivity (MD) in the corpus callosum (CC). Higher nicotinamide adenine dinucleotide dehydrogenase levels correlated with lower volumes of thalamus ($p = .005$) and cerebral white matter ($p = .049$) and, in the CC, with lower FA ($p = .011$, body; $p = .005$, genu; $p = .009$, total CC) and higher MD ($p = .023$, body; $p = .035$, genu; $p = .019$, splenium; $p = .014$, total CC). Greater cytochrome *c* oxidase levels correlated with lower thalamic ($p = .034$) and cerebellar gray matter ($p = .021$) volumes. The results indicate that systemic mitochondrial cellular bioenergetics are associated with brain health in HIV.

Keywords: brain, HIV, mitochondria, oxidative phosphorylation, oxidative stress

Introduction

Approximately 40 million people worldwide are infected with the human immunodeficiency virus (HIV).[1] Many HIV+ individuals demonstrate evidence of abnormal brain morphometry on magnetic resonance imaging (MRI), including thinning of the cerebral cortex and decreased volumes of cortical and subcortical gray matter regions.[2–7] Diffuse microstructural changes in white matter have been revealed by diffusion tensor imaging (DTI).[8–13] Suppressive combination antiretroviral therapy (cART) does not reverse these brain structural alterations,[14–16] even when initiated during primary infection (<12 months after exposure).[17] To devise strategies for patient care, research is needed to delineate the key mechanisms underlying the evolution of brain abnormalities in HIV.

Converging data implicate the impairment of brain mitochondrial dynamics in HIV neuropathogenesis. HIV proteins alter the physiology of mitochondria.[18] For example, neuronal damage, dysfunction, and atrophy can be induced *in vitro*[19,20] by the HIV-1 transactivator of transcription protein (Tat): Tat exposure perturbs mitochondrial oxidative phosphorylation (OXPHOS) enzyme activities,[21,22] changes the morphology of cortical mitochondria,[23] and contributes to altered neuronal synaptic transmission.[22,24] OXPHOS is essential for mitochondrial respiration.[25] Dysfunction of OXPHOS enzymes increases oxidative stress and generation of reactive oxygen species (ROS), activating the intrinsic apoptotic mitochondrial pathway.[26–29] Failure to inhibit this cycle has been linked to neurodegenerative diseases[29,30] and HIV-associated dementia.[31] However, the possible role of mitochondrial OXPHOS in HIV-associated brain structural pathology has not been investigated.

The present study examined regional brain volumes and white matter microstructural integrity (assessed by multimodal neuroimaging) in relation to systemic mitochondrial parameters, that is, protein levels of mitochondrial OXPHOS

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complex I (nicotinamide adenine dinucleotide: ubiquinone oxidoreductase, CI) and complex IV (cytochrome c oxidase, CIV) in peripheral blood mononuclear cells (PBMCs). Brain volumetric analyses focused on regions of interest (ROIs) identified in prior studies as vulnerable to HIV and/or oxidative stress (i.e., pallidum, thalamus, caudate, hippocampus, cerebral white matter, cerebral subcortical gray matter, and cerebellar gray matter[4,5,32–34]). DTI was used to assess microstructural properties of the largest commissural pathway, the corpus callosum (CC), which has previously shown diffusion abnormalities associated with HIV infection.[13,35,36] CI and CIV represent the initial and terminal aspects of the electron transport chain (ETC).[37] HIV-infected individuals who are cART-naïve exhibit increased oxidative damage, mitochondrial DNA (mtDNA) depletion, and decreased activities of CI-CIV in PBMCs.[38] We hypothesized that altered PBMC mitochondrial CI and CIV levels would be associated with lower brain volumes on MRI and with abnormal, DTI-based, white matter microstructural indices.

Methods

Study sample

Protein levels of OXPHOS CI and CIV were obtained at entry in a subset of participants from the Hawaii Aging with HIV Cohort–Cardiovascular Disease (HAHC-CVD) study,[39] a longitudinal study of subclinical cerebro-CVD in HIV-infected individuals on cART. Regional brain volumes were obtained once, cross-sectionally, to correspond to an annual visit of this study: MRI was performed at entry for 19 participants, while scans for the remaining nine were acquired in association with annual visits conducted 1 or 2 years later. Recruitment was conducted through referrals from the Hawaii Center for AIDS, community physicians, community advisory board members, and AIDS service organizations. Inclusion criteria included (1) ≥ 40 years old; (2) documented history of HIV infection; (3) on stable cART ≥ 3 months; (4) English as their primary language; and (5) able to understand and provide informed consent. Exclusion criteria included (1) uncontrolled major affective disorder; (2) active psychosis; (3) recorded loss of consciousness >5 min; (4) pregnancy or breastfeeding; (5) factors precluding MRI (e.g., claustrophobia); and (6) any past or present condition [e.g., central nervous system (CNS) infection, traumatic brain injury, stroke, or substance abuse] that was determined by the evaluating physician to present confounding variables. All study participants underwent a general and focused HIV/neurological history and physical examination. Each participant provided written informed consent. The study was approved by the Institutional Review Board in the Office of Compliance at the University of Hawaii.

PBMC isolation

Blood was collected in EDTA vacutainer tubes. PBMCs were isolated over a Ficoll-Paque gradient and washed three times with phosphate-buffered saline (PBS). An aliquot of cells was counted using trypan blue and a hemocytometer. Cells were then viably cryopreserved at a concentration of 10 million/1 mL freezing media (10% fresh dimethyl sulfoxide/90% heated fetal bovine serum) in 0.5-mL aliquots (5 million cells each) in 1.5–2-mL O-RINGED screw-capped cryovials.

OXPHOS enzymes

CI and CIV protein levels were determined in duplicate by immunoassays, as described elsewhere.[40] Vials of PBMCs were thawed and washed in 0.5 mL of PBS twice before addition of 0.5 mL of ice-cold extraction buffer [1.5% lauryl maltoside, 25 mM HEPES (pH 7.4), 100 mM NaCl, plus protease inhibitors (P-8340; Sigma)]. Samples were mixed gently, kept on ice for 20 min, and microcentrifuged at 16,400 rpm for 20 min at 4°C. Samples were loaded on the immunoassays with equal amounts of total cell protein following established guidelines.[40] CI and CIV levels were quantified using densitometric scanning with a Hamamatsu ICA-1000 reader. Protein level was measured as optical density (OD)/μg of protein $\times 10^3$.

Neuroimaging

MRI was performed on a 3.0 Tesla Philips Medical Systems Achieva scanner using an eight-channel head coil (InVision Imaging, Honolulu). High-resolution, MRI anatomical data were obtained for each subject using a sagittal, 3D, turbo field echo T1-weighted (3D TFE T1W) sequence [echo time (TE)/repetition time (TR) = 3.2 ms/6.9 ms; flip angle 8°; slice thickness 1.2 mm with no gaps between slices; in-plane resolution 1.0 mm^2; field of view (FOV) 256 $\times$ 256 mm^2]. Diffusion-weighted MRI scans were acquired using a single-shot, echo planar imaging (EPI) sequence: 24 cm FOV, TR/TE = 7,859 ms/80 ms, flip angle 90°, 3.0-mm-thick slices, 0-mm gap, SENSE factor = 3.1, maximum slew rate 120mT/m/ms, gradient amplitude 40 mT/m, 96 $\times$ 95 acquisition matrix, 2.5 $\times$ 2.5 mm^2 in-plane resolution, and a variable number of slices determined by head size. One image without diffusion sensitization was obtained (i.e., a T2-weighted b0 image). Diffusion weighting was applied along 15 noncollinear directions evenly distributed over a sphere with a b-factor of 1,000 s/mm^2 and four signal averages to increase the signal-to-noise ratio (SNR). Scan time was 8.6 min.

Regional brain volumes were obtained by processing T1-weighted MRI data with FreeSurfer (version 5.0, https://surfer.nmr.mgh.harvard.edu).[41–44] The process includes skull stripping,[45] intensity normalization,[46] Talairach transformation, subcortical white matter and deep gray matter segmentation,[42,43] and cortical gray/white matter boundary and pial surface reconstruction.[41] Intracranial volume (ICV) was used to correct for differences in head size.[47] Following visual quality control of the surfaces and segmentations, volumetric data were determined from the left and right hemispheres for seven ROIs known to be affected by HIV: pallidum, hippocampus, thalamus, caudate, cerebral subcortical gray matter, cerebellar gray matter, and cerebral white matter.[4,13,34,48–52] Total regional volumes were computed by summing over the left and right brain hemispheres.

Diffusion data were processed using DTI protocols, as previously described.[53] In brief, the FSL *eddy_correct* tool was used to correct for motion- and eddy current-induced distortions. To correct for EPI-induced susceptibility artifacts, each subject's b0 image was nonlinearly warped to the corresponding anatomical T1-weighted image. The *dtifit* command in FSL was used to estimate diffusion tensors from the preprocessed images and to obtain maps of the DTI scalar metrics, fractional anisotropy (FA) and mean diffusivity (MD). The FA image corresponding to the Johns Hopkins

University (JHU) Eve atlas was registered to each individual FA scan. The warps were applied to the white matter labels that defined the ROIs in the CC. FA and MD were derived for the genu, body, and splenium of the CC. Each subregion and the total CC were included in the DTI analyses.

Statistical analyses

Descriptive statistics were computed for patient demographics, clinical parameters, PBMC CI and CIV protein levels, regional brain volumes, and DTI indices. Multiple linear regression examined associations between neuroimaging indices and CI and CIV levels (separately). Analyses of DTI metrics focused on FA and MD of the genu, body, splenium, and total CC.

Sensitivity analyses were conducted for both brain volume and DTI models to evaluate potential contributions from additional variables, including age, sex, ICV, CD4 nadir, years since HIV diagnosis, years on cART, substance use, and use of any of the three nucleoside reverse transcriptase inhibitors (NRTIs): azidothymidine (AZT), stavudine (d4T), or didanosine (ddI). Age was the only covariate retained in the final regression models that examined DTI metrics. ICV was utilized as a covariate in analyses of regional brain volumes. Significance was defined by $p < .05$, with $0.05 \leq p < .1$ considered indicative of a trend. Volumes and DTI measurements were assessed for normality through visual inspection of histograms and the Shapiro–Wilk test. Regression diagnostics for all models were examined for violations. SPSS 22 and SAS, v9.4 (SAS Institute, Inc., Cary, NC), were used for statistical analyses.

Results

PBMC CI and CIV levels and neuroimaging data were available for 28 HIV+ individuals who were all on stable cART and between the ages of 40 and 70 [mean age 52 ± 7 years; predominantly male (86%); mean duration of HIV infection $= 20.5 \pm 7.0$ years; mean duration of cART $= 18.1 \pm 5.9$ years]. Plasma HIV RNA was undetectable (<50 copies/mL) in 23 (82%) of the study participants and the remaining five subjects had a median HIV RNA count of 180 copies/mL (range: 53–6,280). CI values ranged from 15 to 56 OD/$\mu g \times 10^3$, while the range of CIV was narrower (11–35 OD/$\mu g \times 10^3$). The median (interquartile range) time from the blood draw to MRI was 2 (1–24) months. Demographic and clinical data are summarized in Table 1 and regional brain volumes and DTI measures (FA and MD) presented in Table 2. CI and CIV levels were not associated with substance use variables or with history of treatment with AZT, d4T, or ddI.

Table 1. Study Sample: Demographics, Clinical Variables, and Peripheral Blood Mononuclear Cell Oxidative Phosphorylation Protein Levels

Characteristics	
N	28
Age (years)	52.5 ± 7.2
Sex (male)	24 (85.7%)
Race/ethnicity (Caucasian)	13 (46.4%)
Years since HIV diagnosis	20.5 ± 7.0
Years on cART	18.1 ± 5.9
CD4 count (cells/mm^3)	501 ± 203
Nadir CD4 count (cells/mm^3)	176 ± 144
Undetectable plasma HIV RNA (<50 copies/mL)	23 (82.1%)
PBMC OXPHOS Complex 1 protein level (OD/$\mu g \times 10^3$)	33.8 ± 9.4
PBMC OXPHOS Complex 4 protein level (OD/$\mu g \times 10^3$)	27.6 ± 5.6
Ever used AZT, d4T, or ddI	18 (64.3%)
Ever used any drug[a]	25 (89.3%)
Marijuana use (lifetime frequency)[b]	
Never	0 (0%)
1–10 times	7 (28%)
>10 times	18 (72%)
Methamphetamine use (lifetime frequency)[b]	
Never	15 (60%)
1–10 times	3 (12%)
>10 times	7 (28%)
Use of stimulants (lifetime frequency)[b]	
Never	20 (80%)
1–10 times	2 (8%)
>10 times	3 (12%)
Alcohol use[c]	
Never	11 (42.3%)
Sometimes (≤4 times/month)	11 (42.3%)
Frequently (>2 times/week)	4 (15.4%)

Data are given as mean ± SD for continuous variables and *n* (%) for categorical variables.

[a]Drugs include marijuana, cocaine, crack, stimulants, phencyclidine, methamphetamine, heroin, lysergic acid diethylamide, ecstasy, nitrates, glue, ketamine, methadone, barbiturates, painkillers, and sedatives.

[b]$n = 25$.

[c]$n = 26$.

AZT, azidothymidine; cART, combination antiretroviral therapy; d4T, stavudine; ddI, didanosine; OD, optical density; OXPHOS, oxidative phosphorylation; PBMCs, peripheral blood mononuclear cells; SD, standard deviation.

PBMC OXPHOS CI and CIV protein levels and regional brain volumes

After controlling for ICV, we found associations ($p < .05$) between higher CI protein levels and smaller thalamic and cerebral white matter volumes (Table 3). Only the former relationship was significant after Bonferroni correction for multiple comparisons ($p < .007 = .05/7$). Volumes of cerebral subcortical gray matter, cerebellar gray matter, pallidum, and hippocampus showed trend-level inverse associations with CI protein levels ($p < .10$). Significant associations were observed between higher CIV levels and lower cerebellar gray matter and thalamic volumes independently of ICV, although neither survived multiple comparison correction.

When regression analyses were restricted to the 23 participants with undetectable plasma HIV RNA, the CI associations with regional volumes became stronger and more significant ($\beta = -0.51$, $p = .002$ for thalamus; $\beta = -0.32$, $p = .039$ for cerebral white matter). Similarly, CIV was associated with thalamic volume ($\beta = -0.39$, $p = .025$), and the relationship between CIV and cerebellar gray matter was strong enough to survive Bonferroni correction ($\beta = -0.55$, $p = .001$).

PBMC OXPHOS CI and CIV protein levels and DTI metrics

After adjustment for age, higher CI protein levels were significantly associated with lower FA in the total CC, genu,

Table 2. Regional Brain Volumes, Intracranial Volume, and Diffusion Tensor Imaging-Derived Fractional Anisotropy and Mean Diffusivity for the Corpus Callosum

Brain region	*Volume (mm^3)*
Pallidum	3,319 ± 371
Hippocampus	7,970 ± 762
Cerebral subcortical GM	77,316 ± 5,986
Cerebellar GM	87,493 ± 8,737
Thalamus	12,913 ± 1,182
Caudate	7,188 ± 731
Cerebral WM	488,178 ± 54,007
ICV (in 10^3 mm^3)	1,455.10 ± 277.09
Brain region	*FA*
Genu of CC	0.50 ± 0.04
Body of CC	0.47 ± 0.04
Splenium of CC	0.55 ± 0.03
Total CC	0.51 ± 0.03
Brain region	*MD*
Genu of CC	$9.5 \times 10^{-4} \pm 9.2 \times 10^{-5}$
Body of CC	$1.1 \times 10^{-3} \pm 9.0 \times 10^{-5}$
Splenium of CC	$9.9 \times 10^{-4} \pm 7.2 \times 10^{-5}$
Total CC	$9.9 \times 10^{-4} \pm 7.6 \times 10^{-5}$

Mean values ± SDs reported for all variables; $n = 28$.
CC, corpus callosum; FA, fractional anisotropy; GM, gray matter; ICV, intracranial volume; MD, mean diffusivity; WM, white matter.

and body after Bonferroni correction ($p < .013 = .05/4$). A trend was observed between increased CI and decreased FA in the splenium ($p < .10$) (Table 4). Similarly, we found a trend-level association between higher CIV protein levels and lower FA in the splenium. The CIV level was not associated with FA in other callosal regions (Table 5).

Higher levels of CI were linked to increased MD in the genu, body, splenium, and total CC (Table 4), although the associations were not significant when Bonferroni corrected. CIV levels did not relate to MD in the genu, body, or total CC. A trend toward a positive correlation between CIV and MD was noted in the splenium (Table 5).

CI and CIV associations with DTI metrics increased in strength and significance when only the participants with undetectable plasma HIV RNA were considered. CI was significantly related to FA in the total CC ($\beta = -0.70$, $p < .001$) and all subregions (e.g., $\beta = -0.63$, $p = .002$, for splenium) after correction for multiple comparisons. Notably, all associations between CI and MD survived Bonferroni correction (e.g., $\beta = 0.62$, $p < .001$, for total CC). CIV showed a trend relationship to FA in the genu ($\beta = -0.38$, $p = .074$) and was linked to MD in the splenium ($\beta = 0.44$, $p = .021$) and total CC ($\beta = 0.34$, $p = .068$).

Discussion

Results from this study provide the first examination of systemic OXPHOS CI and CIV levels and neuroimaging metrics in HIV+ individuals. A higher PBMC CI level was significantly associated with lower thalamic volume after correction for multiple comparisons. CI was also inversely related to cerebral white matter volume and showed trends toward similar relationships with volumes of cerebral subcortical gray matter, cerebellar gray matter, hippocampus, and pallidum. A higher CIV level was associated with lower volumes of cerebellar gray matter and thalamus. These results are supported by well-known differences in brain regional sensitivity to oxidative stress. The thalamus is selectively vulnerable to neurodegeneration induced by impaired oxidative metabolism.[54,55] Neurons in the cerebellar granule layer (unlike those in the cerebral cortex) are highly susceptible to oxidative stress and consequent cell death, as are hippocampal CA1 neurons.[33] In earlier work, we identified strong associations between increased PBMC levels of the ROS-induced lesion 8-oxo-2′-deoxyguanosine (8-oxo-dG), a marker of mtDNA oxidative damage,[56] and reduced brain volumes in an HIV+ sample that included all participants of the current study.[32] HIV alters mitochondrial morphology,[23] physiology,[18] and respiratory dynamics.[57] Our study suggests that such mitochondrial changes exert an adverse impact on brain structure.

Mitochondrial dysfunction may underlie multiple aging-related and neurodegenerative pathologies[58] such as Alzheimer's disease,[59] Parkinson's disease,[60] and HIV-associated neurocognitive disorders (HANDs).[61] Examination of frontal cortex autopsy tissue from patients with HANDs has revealed significant mitochondrial abnormalities: increased mtDNA 8-oxo-dG damage,[62] accumulated mtDNA mutations and deletions,[62] and dysregulated mitochondrial fission and fusion.[63] Reduced mitochondrial biogenesis and increased neuroinflammation were recently identified in frontal cortices of cART-treated HAND donors.[64] ROS-induced oxidative DNA damage may be crucial in HIV-related neurodegenerative processes.[62]

The etiology of HIV-related mitochondrial dysfunction is likely to be multifactorial. Prior studies implicate nucleoside NRTIs[65–68]; however, no association was observed in the present study between treatment history with AZT, d4T, or ddI and mitochondrial complex protein levels. Other work has focused on deleterious effects of substance use on mitochondrial function in HIV. Methamphetamine is especially disruptive,[69,70] particularly in the setting of HIV infection.[71] The lack of correlation between substance use histories and CI and CIV levels in the current study suggests that mitochondrial function may be more strongly linked to the HIV disease process. HIV proteins and disease dynamics disrupt mitochondrial integrity and potentiate the apoptotic pathway.[72–75]

Also identified in our study were relationships between increased PBMC complex levels and reduced microstructural integrity of brain white matter. Higher CI levels corresponded to significantly reduced FA and increased MD within the genu, body, and entire CC and at trend level in the splenium. Trend associations of higher CIV levels with decreased FA and increased MD were noted in the splenium. Relationships between DTI measures and CIV (unlike CI) may have failed to reach significance because of the more restricted range of CIV levels. Progressively diminishing mitochondrial respiratory chain dysfunction has been reported with the movement of electrons down the ETC: a study of cART-naïve HIV-infected patients found that the

Table 3. Associations Between Regional Brain Volumes and Levels of Peripheral Blood Mononuclear Cell Complex I and Complex IV by Multiple Regression Controlling for Intracranial Volume

Brain region (volume)	*Predictor variables*	β	p	*Adjusted R^2*
Pallidum	CI level	−0.32	.091	
	ICV	0.40	.040	
				0.14
Hippocampus	CI level	−0.31	.095	
	ICV	0.45	.020	
				0.17
Cerebral subcortical GM	CI level	−0.30	.054	
	ICV	0.70	<.001	
				0.44
Cerebellar GM	CI level	−0.33	.069	
	ICV	0.48	.012	
				0.21
Thalamus	CI level	−0.46	**.005***	
	ICV	0.62	<.001	
				0.43
Caudate	CI level	−0.33	.864	
	ICV	0.36	.071	
				0.06
Cerebral WM	CI level	−0.27	**.049**	
	ICV	0.78	<.001	
				0.55
Brain region (volume)	*Predictor variables*	β	p	*Adjusted R^2*
Pallidum	CIV level	−0.07	.708	
	ICV	0.31	.128	
				0.04
Hippocampus	CIV level	−0.20	.308	
	ICV	0.33	.092	
				0.11
Cerebral subcortical GM	CIV level	−0.22	.159	
	ICV	0.58	.001	
				0.40
Cerebellar GM	CIV level	−0.42	**.021**	
	ICV	0.30	.088	
				0.27
Thalamus	CIV level	−0.36	**.034**	
	ICV	0.43	.013	
				0.34
Caudate	CIV level	0.004	.984	
	ICV	0.36	.077	
				0.06
Cerebral WM	CIV level	−0.15	.286	
	ICV	0.68	<0.001	
				0.50

CI and CIV levels are measured as OD/μg of protein $\times 10^3$. Volumes are in mm^3. p-Values <.05 are shown in *bold*, and standardized β-values are presented along with the model's adjusted R^2; $n = 28$.

*Significant after Bonferroni correction for multiple comparisons.

CI, complex I; CIV, complex IV; ICV, intracranial volume; OD, optical density.

activities of respiratory chain complexes II, III, and IV were reduced by 41%, 38%, and 19%, respectively, compared with HIV-negative controls.[38]

DTI studies of HIV+ adults have reported compromised microstructural integrity of the CC; for example, FA reductions in the splenium (relative to HIV-negative controls), which were associated with diminished neurocognitive functioning.[13] Anterior callosal thinning has been detected in HIV+ individuals and linked to T cell decline.[76] Results of the current investigation are consistent with published HIV research; moreover, this is the first study to link PBMC CI and CIV protein levels to FA and MD in the CC. The observed connection between PBMC complex levels and callosal DTI metrics constitutes evidence that systemic mitochondrial dysfunction may contribute to reduced microstructural brain integrity in HIV.

It is worth noting that our participants were chronically infected and on cART for an average of 18 years. PBMC CI and CIV levels corresponded to neuroimaging measures independently of cART regimen or age. Interestingly, these associations were stronger in individuals who were virally suppressed: long-term damage and oxidative disruption may be more clearly delineated when not masked by variability

Table 4. Associations Between Diffusion Tensor Imaging–Derived Metrics for the Corpus Callosum (Fractional Anisotropy and Mean Diffusivity) and Peripheral Blood Mononuclear Cell Complex I Level by Multiple Regression, Controlling for Age

Brain region (FA)	*Predictor variables*	β	p	*Adjusted R^2*
Body of CC	CI level	–0.47	**.011***	
	Age	–0.18	.307	
				0.22
Genu of CC	CI level	–0.51	**.005***	
	Age	–0.15	.383	
				0.25
Splenium of CC	CI level	–0.35	.072	
	Age	–0.18	.338	
				0.10
Total CC	CI level	–0.48	**.009***	
	Age	–2.04	.241	
				0.24
Brain region (MD)	*Predictor variables*	β	p	*Adjusted R^2*
Body of CC	CI level	0.41	**.023**	
	Age	0.33	.057	
				0.26
Genu of CC	CI level	0.36	**.035**	
	Age	0.46	.008	
				0.33
Splenium of CC	CI level	0.41	**.019**	
	Age	0.36	.040	
				0.28
Total CC	CI level	0.42	**.014**	
	Age	0.41	.016	
				0.34

p-Values <.05 are shown in *bold*, and standardized *β*-values are presented along with the model's adjusted R^2. CI level is measured as OD/μg of protein $\times 10^3$; $n = 28$.

*Significant after Bonferroni correction for multiple comparisons.

Table 5. Associations Between Diffusion Tensor Imaging–Derived Metrics for the Corpus Callosum (Fractional Anisotropy and Mean Diffusivity) and Peripheral Blood Mononuclear Cell Complex IV Level by Multiple Regression, Controlling for Age

Brain region (FA)	*Predictor variables*	β	p	*Adjusted R^2*
Body of CC	CIV level	–0.17	.402	
	Age	–0.22	.263	
				0.01
Genu of CC	CIV level	–0.31	.115	
	Age	–0.18	.348	
				0.07
Splenium of CC	CIV level	–0.35	.065	
	Age	–0.18	.332	
				0.11
Total CC	CIV level	–0.30	.123	
	Age	–0.23	.226	
				0.09
Brain region (MD)	*Predictor variables*	β	p	*Adjusted R^2*
Body of CC	CIV level	0.17	.351	
	Age	0.37	.056	
				0.11
Genu of CC	CIV level	0.09	.613	
	Age	0.49	.009	
				0.20
Splenium of CC	CIV level	0.33	.069	
	Age	0.37	.041	
				0.22
Total CC	CIV level	0.23	.197	
	Age	0.44	.018	
				0.21

Standardized *β*-values are presented along with the model's adjusted R^2. CIV level is measured as OD/μg of protein $\times 10^3$; $n = 28$.

due to inclusion of viremic participants in the analyses. As ours was a cross-sectional study, we could not determine whether disrupted OXPHOS was secondary to mitochondrial damage during acute HIV infection or represents an ongoing process that continues despite potent cART. Our PBMC CI and CIV data are in accordance with decreased PBMC mtDNA and functional disruption along the ETC observed in antiretroviral-naïve HIV+ individuals.[38]

Higher levels of CI and CIV can be expected to lead to increased generation of ROS and to cellular injury, including mitochondrial damage. The results presented here are consistent with our previous report that greater mtDNA damage as measured by PBMC mtDNA 8-oxo-dG is associated with regional brain atrophy.[32] Indeed, in our cohort, we see a positive correlation between CI levels and mtDNA 8-oxo-dG levels (Gangcuangco LMA *et al.*, in review).

In the same manuscript under review, we also demonstrate that CI levels in our HIV-infected participants were lower (not higher) compared with HIV-negative control participants of similar age and gender. Lower CD4 count and higher levels of circulating proinflammatory cytokines are known risk factors for HIV-related CNS pathology and correlated with lower CI and CIV protein levels within the HIV+ group. We hypothesize that these results, which at first glance appear inconsistent with our present study findings, may be secondary to the measurement of protein levels and not the functional capacity of the OXPHOS system. It is possible that HIV is associated initially with impaired OXPHOS function before decreases in protein levels can be detected. A compensatory increase in OXPHOS may lead to enhanced ROS levels, which damage DNA, lipids, proteins, and membrane permeability within mitochondria.[77] CI, in particular, has been identified as a common site of superoxide generation.[77,78] Increased ROS production can be a consequence of functional alterations such as stoichiometric mismatches in the ETC complexes. Such mismatches result in longer residence time of electrons on sites of complexes that mediate electron reduction of O_2^-, resulting in increased production of H_20_2 and superoxide.[27]

Important limitations of the present work merit discussion. Our study involved cross-sectional examination of PBMC OXPHOS levels rather than mitochondrial assessments in the CNS, and the CI/CIV protein level, but not activity, was measured. It is possible that inclusion of CNS markers or enzymatic activities would yield additional associations. CI/CIV protein and activity levels are highly correlated,[79] and our results indicate that mitochondrial OXPHOS protein levels in PBMCs account for considerable variance in neuroimaging indices. Still, the adjusted R^2 values (only moderately large even for models showing significant associations) suggest the influence of unmeasured factors. Additional research must elucidate the causal relationship between systemic mitochondrial PBMC dysfunction and brain alterations, with consideration given to other possible mechanisms by which HIV may affect the brain.

While regional brain volumetric decreases and callosal degradation may promote neurocognitive decline, their impact on functional outcomes was beyond the scope of this paper. Larger comprehensive studies are required to determine the relationships between mitochondrial function, brain structure, and cognitive/behavioral performance in HIV-infected individuals. The interval between blood collection and MRI varied across our participants, and mitochondrial assessments were done in bulk PBMCs and not specifically within lymphocytes or monocytes and macrophages. Given that much of the CNS pathology in HIV infection is believed to be monocyte/macrophage-mediated, it will be important to conduct separate mitochondrial assessments to determine the contributions of each cell type. Finally, our sample size was restricted, although the study was sufficiently powered to identify significant relationships among variables of interest.

In summary, the present study revealed significant associations between PBMC mitochondrial CI and CIV levels and brain imaging markers in chronically infected HIV+ individuals, independently of cART regimen or age. Further research is needed to define the role of mitochondrial dysfunction in development of brain abnormalities in HIV.

Author Disclosure Statement

No competing financial interests exist.

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