Distinct associations of peripheral mtDNA copy number and bioenergetic function with cognition in women with HIV and psychiatric comorbidities

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Background: Women with HIV (WWH) are disproportionately affected by cognitive impairment across multiple domains, with learning and memory consistently among the most vulnerable. Mechanistic investigations into the increased burden of cognitive impairment in people with HIV have highlighted mitochondrial dysfunction as a potential contributor. This study examined associations between two peripheral markers of mitochondrial health, mitochondrial DNA copy number (mtDNAcn) and mitochondrial bioenergetic function, and cognitive function in WWH. We hypothesized that mtDNAcn and mitochondrial function metrics would demonstrate distinct associations with cognitive outcomes, particularly learning and memory.

Methods: Fifty-eight WWH completed 12 neuropsychological tests assessing – verbal learning, verbal memory (recall and recognition), attention, working memory, executive function, fluency, processing speed, and motor function. Peripheral blood mononuclear cells (PBMCs) were isolated, and mitochondrial health was assessed using qPCR (mtDNAcn) and the Seahorse Cell Mito Stress Test (mitochondrial function). Pearson correlations examined associations between mitochondrial health and cognitive domain scores.

Results: Participants were on average 54.9 years old (SD = 8.2), 94.8% were Black, 58.6% had a lifetime diagnosis of major depressive disorder, and 63.7% met criteria for cognitive impairment in $\geq$2 domains. Higher mitochondrial proton leak was significantly associated with worse verbal memory including recall ($r = -0.396$, $P = 0.002$) and recognition ($r = -0284$, $P = 0.031$), as well as poorer working memory ($r = -0.261$, $P = 0.048$). No significant associations were observed between mtDNAcn and cognition.

Conclusions: These findings identify peripheral mitochondrial proton leak as a potential biological correlate of verbal and working memory difficulties in WWH and highlight the importance of assessing multiple dimensions of mitochondrial health when investigating cognitive impairment.

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Introduction

As life expectancy increases among people with HIV (PWH), cognitive impairment remains a significant clinical concern despite effective antiretroviral therapy (ART) [1]. Cognitive impairment affects an estimated 30–50% of PWH compared with approximately 19% among older adults without HIV [2,3]. Women with HIV

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(WWH) appear to be disproportionately affected, with several studies reporting higher rates of cognitive impairment compared with both men with HIV and women without HIV [4–6]. Among the cognitive domains affected, learning and memory are the most consistently vulnerable [6].

Previous mechanistic investigations into the increased burden of cognitive impairment in PWH have highlighted mitochondria as a potential contributor to cognitive impairment [7–10]. Emerging studies have examined mitochondrial DNA copy number (mtDNAcn), a marker reflecting mitochondrial health in terms of mitochondrial genomic content and abundance [11], as a potential biomarker of cognitive impairment in PWH. However, findings have been inconsistent, with some studies reporting that higher mtDNAcn is associated with worse cognitive outcomes [12], while others report the opposite [13,14]. These discrepancies may reflect the inherently dynamic nature of mitochondria and mtDNAcn [15], suggesting that mtDNAcn alone may not fully capture mitochondrial processes relevant to cognitive impairment. Notably, few studies have examined mtDNAcn specifically in WWH.

In addition to mitochondrial genomic content, measures of mitochondrial bioenergetic function may provide complementary insight into mitochondrial health. Functional assessments capture aspects of mitochondrial efficiency and energy production that are not reflected by mtDNAcn alone. Importantly, increased mitochondrial function does not necessarily indicate improved mitochondrial health, as heightened activity may reflect compensatory or inefficient bioenergetic states rather than optimal function [16]. To date, few studies have examined mitochondrial function in relation to cognitive impairment in PWH and none have investigated these relationships in WWH. One study reported an association between increased mitochondrial superoxide levels, used as a proxy for mitochondrial dysfunction, and worse cognitive performance, although only a small number of WWH were included ($n = 9$) and functional assessments were limited [17].

Evidence from other disease populations further suggest that peripheral mitochondrial function may be associated with cognitive outcomes. For example, higher levels of mitochondrial proton leak, an indicator of inefficient oxidative phosphorylation, have been linked to poorer cognition [16]. Similarly, preclinical studies have demonstrated that mitochondrial proton leak impairs learning in mice [18,19]. However, whether similar relationships between mitochondrial bioenergetic function and cognition exist in WWH remains unknown. Understanding these relationships is particularly important given the elevated inflammatory burden and oxidative stress associated with HIV infection, both of which can disrupt mitochondrial function [20–22]. Additionally, biological mechanisms associated with female sex may influence both mitochondrial biology and cognitive vulnerability [6,23–26].

Although mtDNAcn and mitochondrial bioenergetic function both reflect aspects of mitochondrial health, they represent distinct biological processes and may contribute differently to cognitive outcomes. However, their relationship and potential contributions to cognition have not been examined in WWH. Thus, the present study investigated relationships among peripheral mtDNAcn, mitochondrial bioenergetic function, and cognitive performance in WWH. We hypothesized that mtDNAcn and mitochondrial function would demonstrate distinct associations with cognitive outcomes and that mitochondrial dysfunction, particularly increased proton leak, would be associated with worse learning and memory performance in WWH.

Methods

Participants

This work leveraged samples from participants enrolled in the Johns Hopkins Brain Health Program, originally recruited for a larger clinical study investigating cognition in cognitively impaired WWH. Participants were women aged 18–65 years with confirmed HIV infection, stable ART, and viral suppression (plasma HIV RNA <1000 copies/ml, verified via laboratory testing and medication review). Additional inclusion criteria included: English as a first language; ability to provide informed consent and attend study visits; elevated self-reported stress, defined as a score >14 on the Perceived Stress Scale (PSS)-10 [27] and/or a current mood, anxiety, or stress/trauma-related disorder based on the Structured Clinical Interview for DSM (SCID)-5; and evidence of objective cognitive impairment, defined as impairment in at least one cognitive domain on the Women's Interagency HIV Study (WIHS) neuropsychological test battery [28,29]. Demographically adjusted normative equations from women without HIV in the WIHS were applied to determine cognitive impairment. The study was approved by the Johns Hopkins Institutional Review Board and all participants provided written informed consent.

Exclusion criteria were: current use of hormone-based contraceptives (e.g., oral contraceptives or transdermal patch); pregnancy, postpartum, or lactation; current regular use of corticosteroids; history of closed head injury with loss of consciousness exceeding 1 h; current diagnosis of schizophrenia or schizoaffective disorder; untreated hypertension or diabetes; history of dementia or other central nervous system (CNS) or AIDS-defining neurologic conditions; current substance use disorder within the past 6 months; and positive urine toxicology screen or breathalyzer at study visits, with the exception of cannabis.

Eligible participants provided a blood sample and completed all cognitive assessments. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and cryopreserved at −80°C in RPMI-1640 medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 1% penicillin-streptomycin (100 U/ml penicillin and 100 μg/ml streptomycin) and 10% dimethyl sulfoxide (DMSO).

Cognitive testing

Based on preclinical research demonstrating a mechanistic link between mitochondrial function and memory impairments outside of HIV infection [30], and prior work identifying memory as a particularly vulnerable domain in WWH [31], the primary cognitive outcomes in this study were verbal learning, delayed verbal recall, verbal recognition, and working memory. Verbal learning, delayed verbal recall, and verbal recognition, which represent distinct aspects of episodic verbal memory, were assessed using the Hopkins Verbal Learning Test-Revised (HVLT-R) [32]. Working memory, representing the temporary storage and manipulation of information, was measured via the Letter-Number Sequencing test [33].

Secondary cognitive outcomes included measures of executive function [Trail Making Test (TMT)-Part B [34], Stroop [35]-interference trial], attention and processing speed (TMT-Part A, Stroop-color, Symbol Digit Modality Test [36]), verbal fluency (letter and animal fluency [37]), and motor function (Grooved Pegboard [38], dominant and nondominant hand). All assessments were administered using validated neuropsychological tests. Domain level scores were utilized instead of individual cognitive tests to provide a more robust assessment of underlying cognitive constructs. Raw test scores were converted to z-scores based on demographic-adjusted normative data [28,29].

Mitochondrial DNA copy number quantification

PBMCs isolated and cryopreserved as described above were used for mitochondrial DNA copy number (mtDNAcn) analyses. On the day of the assay, PBMCs were rapidly thawed, counted manually using a hemocytometer, and 2.25 million cells were pelleted for analysis. Genomic DNA was obtained using a direct cell lysis approach, which has been shown to yield more consistent mtDNAcn measurements compared to other methods of extraction [39]. Cell pellets were resuspended in 100 μl of QuickExtract DNA Extraction Solution (Lucigen), transferred to PCR tubes, and lysed using a thermocycler protocol of 15 min at 68°C followed by 10 min at 95°C.

Lysates were centrifuged at 17 000*g* for 15 min at room temperature to remove debris. The resulting supernatant, containing genomic and mitochondrial DNA, was transferred to clean microcentrifuge tubes and diluted 1:30 for quantitative PCR (qPCR) analysis. Relative mtDNA content was assessed by quantitative PCR (qPCR) targeting the mitochondrial-encoded *ND1* gene. Nuclear-encoded *RPPH1* and *Beta-Globin* genes served as reference genes for normalization. Primer sequences for all targets are provided in Table 1, Supplemental Digital Content, https://links.lww.com/QAD/D953. qPCR was performed using SYBR green chemistry on a QuantSTudio Flex 6 (Applied Biosystems) under standard cycling conditions [40]. Estimated mtDNA copies per cell were derived by multiplying relative mtDNA values by two, assuming two copies of nuclear DNA per diploid cell. This approach provides an estimate of mtDNA content normalized to nuclear DNA rather than absolute mtDNAcn and does not rely on external standard curves [41,42].

Mitochondrial bioenergetic function: Seahorse Cell Mito Stress Test

Mitochondrial bioenergetic analyses were performed using PBMCs isolated as described above, using the same samples used for mtDNAcn analyses. The day prior to the assay, Seahorse XFe24 sensor cartridges were hydrated in Seahorse XF calibrant solution according to the manufacturer's instructions (Agilent Technologies). Seahorse XFe24 cell culture microplates were coated with Cell-Tak cell adhesive (22.5μg/ml, Corning) to facilitate cell adherence.

On the day of the assay, cryopreserved PBMCs were rapidly thawed and counted manually using a hemocytometer. Cell pellets were resuspended in Seahorse XF assay medium (Agilent Technologies) to achieve a concentration of 750 000 cells per 100 μl. A total of 750 000 cells in 100 μl were seeded per well in the coated Seahorse plate. Plates were centrifuged at 200*g* for 1 min (acceleration rate: 5; brake: 0), rotated 180°, and centrifuged again under identical conditions. Subsequently, 400 μl of fresh prewarmed Seahorse assay medium was added to each well, and the plate incubated for 30 min at 37°C in a non-CO_2 incubator.

Mitochondrial stress test compounds were reconstituted according to the manufacturer's guidelines to final working concentrations of 2 μM oligomycin, 2 μM FCCP, and 1.5 μM each of rotenone and antimycin A. The assay yielded seven mitochondrial function outcomes, including basal respiration, maximum respiration, nonmitochondrial respiration, ATP production, spare respiratory capacity, percentage coupling efficiency, and proton leak.

Statistical analyses

Demographic and clinical characteristics were summarized using descriptive statistics. Prior to primary analyses, potential confounders were evaluated *a priori* based on biological plausibility and previous literature, including age, menopausal status, substance use, and HIV-related clinical variables. Covariates were retained only if they met prespecified criteria for confounding by materially altering the estimated association between the predictor

and outcome. As no covariates met these criteria, unadjusted linear regression models were utilized. Variables were inspected for normality and outliers prior to analyses.

Associations between mitochondrial measures and cognition were assessed using two-sided Pearson's correlations between mitochondrial outcomes and domain-specific cognitive performance measures. HVLT-R (total learning, delayed verbal recall, verbal recognition) and letter-number sequencing outcomes (working memory condition) were designated as primary cognitive outcomes and were evaluated without correction for multiple comparisons. Additional cognitive domains were examined as secondary outcomes, and false discovery rate (FDR) correction was applied to control for multiple comparisons.

To characterize latent structure within mitochondrial bioenergetic function and reduce dimensionality of Seahorse-derived functional outcomes, a principal component analysis (PCA) with varimax rotation was conducted across the seven mitochondrial function outcomes. PCA yielded a two-component solution. The first component accounted for 67.41% of the variance and the second accounted for 17.93%. Loadings indicated that the first component reflected general mitochondrial bioenergetic function, whereas the second component was primarily characterized by proton leak (Table 2, Supplemental Digital Content, https://links.lww.com/QAD/D953). The full variance explained by each component and the scree plot are provided in the Supplementary Materials (Table 3, Supplemental Digital Content, https://links.lww.com/QAD/D953; Figure 1, Supplemental Digital Content, https://links.lww.com/QAD/D953). Factor scores were extracted for each participant and retained as latent indicators of mitochondrial bioenergetic function. Associations between mitochondrial PCA component scores and cognitive performance were then assessed using Pearson correlations.

To examine relationships between mitochondrial genomic content and mitochondrial bioenergetic function, associations between mtDNAcn and seahorse-derived mitochondrial function component scores were evaluated. Correlations were first examined between mtDNAcn and PCA-derived mitochondrial function component scores, followed by analyses examining associations between mtDNAcn and individual seahorse metrics. All analyses were conducted in R (version 4.3.3).

Table 1. Sociodemographic and clinical characteristics of study sample of women with HIV (WWH).

	WWH ($n=58$)
Demographics	
Age, *M* (SD)	54.9 (8.2)
Years of education, *M* (SD)	11.9 (2.0)
WRAT reading subtest, *M* (SD)	57.1 (11.4)
Race/ethnicity, *n* (%)	
Black, non-Hispanic	55 (94.8)
White, non-Hispanic	3 (5.2)
Mental and cognitive health	
Lifetime MDD diagnosis, *n* (%)	34 (58.6)
Lifetime PTSD diagnosis, *n* (%)	20 (34.4)
Impaired on 2 or more cognitive domains, *n* (%)	37 (63.7)
Antidepressant medication use, *n* (%)	34 (58.6)
Substance use	
Tobacco use, *n* (%)	
Recent/current	26 (44.9)
Former	22 (39.9)
Never	10 (17.2)
Recent heavy alcohol use, *n* (%)	11 (18.9)
Marijuana use, *n* (%)	
Recent/current	10 (17.2)
Former	24 (41.4)
Never	24 (41.4)
Crack, cocaine, and/or heroin use, *n* (%)	
Recent/current	2 (3.4)
Former	32 (55.2)
Never	24 (41.4)
Clinical factors	
HIV RNA (cp/ml), *n* (%)	
undetectable (<20)	49 (84.4)
>20 and ≤100	8 (13.7)
>100 and <200	1 (1.7)
ARV medications, *n* (%)	
Abacavir	14 (24.1)
Atazanivir	1 (1.7)
Bictegravir	16 (27.6)
Cobicistat	13 (22.4)
Darunavir	5 (8.6)
Dolutegravir	22 (39.9)
Elvitegravir	9 (15.5)
Emtricitabine	40 (68.9)
Lamivudine	14 (24.1)
Raltegravir	4 (6.8)
Rilpivirine	5 (8.6)
Ritonavir	3 (5.2)
Tenofovir alafenamide	39 (67.2)
Tenofovir disoproxil fumarate	2 (3.4)
Menopausal status, *n* (%)	
Reproductive	5 (8.6)
Early transition	1 (1.7)
Medication/other ammenorrhea	16 (27.6)
Late transition	6 (10.4)
Postmenopausal (<2 years)	0 (0.0)
Postmenopausal (2–3 years)	2 (3.4)
Postmenopausal (3–6 years)	2 (3.4)
Postmenopausal (>6 years)	26 (44.9)

Participant characteristics are presented for the full cohort ($n=58$). Continuous variables are reported as mean ± standard deviation (SD), and categorical variables are reported as *n* (%).

Results

Participant characteristics

Table 1 presents the sociodemographic and clinical characteristics of the study sample ($n=58$). Participants had a mean age of 54.9 years (SD = 8.2) and most identified as Black (94.8%). Nearly all participants had undetectable viral loads (84.4%). Over half of the sample met criteria for a lifetime diagnosis of major depressive disorder (58.6%) and 34.4% met criteria for lifetime

Table 2. Correlations between mtDNAcn and cognitive performance.

Outcome measures	mtDNA copies per cell	
	r	*P*-value
Primary		
Learning	0.10	0.49
Memory – recall	0.16	0.24
Memory – recognition	0.08	0.54
Working memory	0.26	0.06
Secondary		
Attention	0.15	0.30
Executive function	0.29	0.04*
Verbal fluency	0.04	0.79
Motor function	-0.10	0.46
Processing speed	0.17	0.22

Pearson correlation coefficients (*r*) and corresponding *P* values are shown for associations between mtDNAcn and cognitive domain *z*-scores. Significant associations prior to FDR correction are reported.

posttraumatic stress disorder. Cognitive impairment in two domains was present in 63.7% of participants.

Associations between mtDNA copy number and cognitive outcomes

The median estimated mtDNAcn per cell was 136.9, with a range of 32.1–415.2 mtDNA copies per cell. mtDNAcn was not significantly associated with learning or memory performance. A positive association was observed between mtDNAcn and executive function, indicating that higher mtDNAcn was associated with better executive functioning ($r=0.29$, $P=0.036$). However, this association did not remain significant after FDR correction ($r=0.29$, $P=0.18$). No other significant associations between mtDNAcn and cognitive outcomes were observed (Table 2).

Associations between mitochondrial function and cognitive outcomes

Descriptive statistics for individual seahorse-derived mitochondrial respiration parameters are provided in the Supplemental Materials (Table 4, Supplemental Digital Content, https://links.lww.com/QAD/D953). Pearson correlations between mitochondrial component scores and cognitive performance outcomes are presented in Table 3. Higher scores on the second mitochondrial component, reflecting greater mitochondrial proton leak, were significantly associated with worse verbal memory performance, including delayed verbal recall ($r=-0.396$, $P=0.002$) and verbal recognition ($r=-0.284$, $P=0.031$), as well as poorer working memory performance on LNS ($r=-0.261$, $P=0.048$). No other significant associations were observed between mitochondrial components and cognitive outcomes. Figure 1 depicts the relationships between the proton leak component and the primary cognitive outcomes.

Relationship between mtDNAcn and mitochondrial bioenergetic function

mtDNAcn per cell was not significantly associated with either PCA-derived mitochondrial component (1 – *general mitochondrial bioenergetic function*: $r=-0.23$, $P=0.094$; 2 – *proton leak*: $r=-0.05$, $P=0.678$). Given the trend toward an association with the general bioenergetic function component, additional analyses examined relationships between mtDNAcn and individual seahorse-derived mitochondrial respiration metrics (Table 5, Supplemental Digital Content, https://links.lww.com/QAD/D953). Higher mtDNAcn was significantly associated with lower maximal respiration ($r=-0.277$, $P=0.029$) and reduced spare respiratory capacity ($r=-0.293$, $P=0.022$) (Figure 2, Supplemental Digital Content, https://links.lww.com/QAD/D953). No significant associations were observed between mtDNAcn and basal respiration, ATP-production, coupling efficiency, non-mitochondrial respiration, or proton leak.

Discussion

This study provides evidence that distinct peripheral mitochondrial markers are differentially associated with cognitive performance in virally suppressed WWH with cognitive impairment. Whereas mtDNAcn showed only modest and domain-specific associations with cognition, mitochondrial bioenergetic function demonstrated strong relationships with both verbal and working memory performance. Specifically, increased mitochondrial proton leak was associated with poorer verbal recall, verbal recognition, and working memory. In addition, higher mtDNAcn was associated with reduced maximum respiration and spare respiratory capacity, suggesting a complex relationship between mitochondrial content and functional capacity in WWH. Together, these findings suggest that mitochondrial genomic content and mitochondrial bioenergetic function capture different aspects of mitochondrial health and may relate differently to cognitive outcomes in WWH.

Mitochondrial functional measures demonstrated the strongest relationships with cognition in this study. Greater mitochondrial proton leak was associated with poorer verbal and working memory performance, particularly delayed verbal recall and recognition as well as poorer working memory. Proton leak reflects reduced efficiency of the mitochondrial electron transport chain and is often considered an indicator of mitochondrial dysfunction. Increased proton leak can arise from oxidative damage to mitochondrial membranes which disrupt the proton gradient required for ATP production. Oxidative stress has been consistently observed in PWH, even in virally suppressed individuals, and has been linked to mitochondrial dysfunction in immune cells [43,44]. Our findings therefore suggest that peripheral mitochondrial dysfunction associates

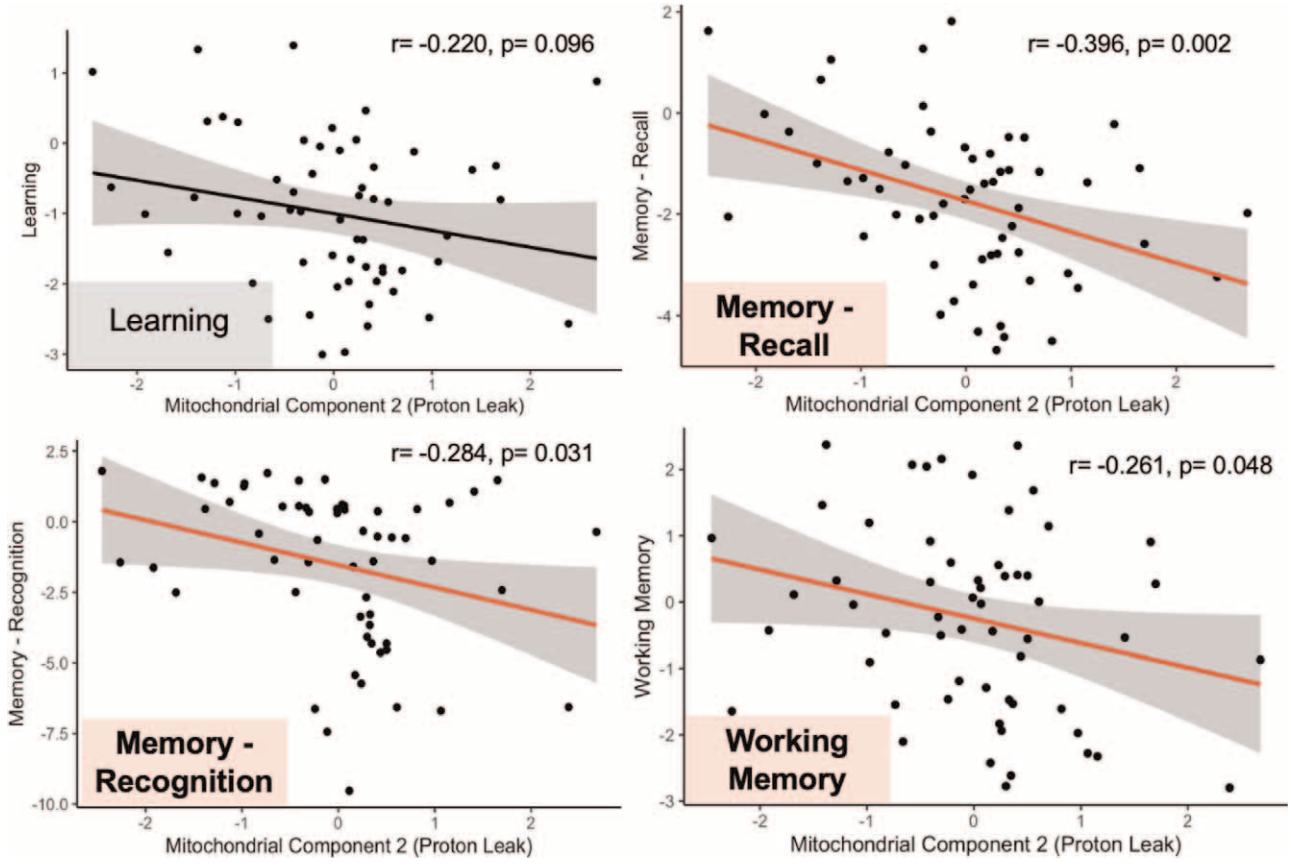

Fig. 1. Elevated proton leak-related mitochondrial function is associated with worse cognitive performance on recall, recognition, and working memory. Scatterplots depict Pearson correlations between the second mitochondrial component, defined primarily by proton leak, and primary cognitive performance measures, including learning, recall, recognition, and working memory. Individual points represent participants ($n = 58$) and shaded areas reflect 95% confidence intervals.

with impairments in memory-related cognitive processes and future assessment of potential contributions from oxidative stress may provide a mechanistic link. Importantly, we did not observe associations between the mitochondrial component reflecting overall respiratory capacity. This pattern suggests that memory performance may be more sensitive to markers of mitochondrial damage, such as proton leak rather than general mitochondrial activity.

In contrast, mtDNAcn demonstrated limited associations with cognitive performance. mtDNAcn was not associated with learning or memory, domains that are frequently impaired in WWH, suggesting that mitochondrial genomic content alone may not capture the mitochondrial processes most relevant to these specific cognitive domains. We observed a modest positive association between higher mtDNAcn and executive function; however, this relationship did not remain significant after correction for multiple comparisons and should be interpreted cautiously. Consistent with previous studies in PWH, mtDNAcn levels in our cohort were lower than those reported in HIV-uninfected populations [45], supporting previous evidence that HIV infection and/or ART treatment may impact mitochondrial biology even in the context of viral suppression. Importantly, mtDNAcn is highly dynamic and responsive to both acute and chronic stress [15], which may limit its utility as a stable biomarker when assessed at a single timepoint.

Taken together, these findings highlight important biological differences between mitochondrial bioenergetic function and mitochondrial genomic content. Proton leak reflects mitochondrial efficiency and functional bioenergetic capacity whereas mtDNAcn reflects mitochondrial abundance and may represent adaptive cellular responses to energetic or metabolic stress. These differences likely explain the distinct cognitive relationships observed in this study, with mitochondrial proton leak demonstrating stronger relationships with memory performance and mtDNAcn showing limited domain-specific associations. In addition, the observed association between higher mtDNAcn and reduced maximal respiration and spare respiratory capacity raises the possibility that increased mtDNAcn may reflect compensatory mitochondrial responses rather than enhanced mitochondrial function. While mtDNAcn and bioenergetic function were modestly associated, these measures

Table 3. Associations between mitochondrial proton leak (component 2) and cognitive function in WWH.

	Mitochondrial component			
	1 – General mitochondrial bioenergetic function		2 – Proton leak	
Outcome measures	*r*	*P*-value	*r*	*P*-value
Primary				
Learning	0.05	0.70	−0.22	0.096
Memory – recall	0.05	0.71	**−0.40**[a]	**0.002**
Memory – recognition	0.15	0.27	**−0.28**[b]	**0.03**
Working memory	0.07	0.59	**−0.26**[b]	**0.048**
Secondary				
Attention	0.04	0.75	−0.19	0.15
Executive function	0.03	0.81	0.01	0.97
Fluency	−0.11	0.42	−0.06	0.65
Motor	0.07	0.58	0.07	0.59
Processing speed	0.04	0.77	−0.13	0.31

Pearson correlation coefficients (*r*) and corresponding *P* values are presented for associations between mitochondrial principal component scores derived from Seahorse bioenergetic parameters and cognitive domain *z*-scores. Component 1 reflects overall mitochondrial respiratory function, whereas Component 2 primarily reflects mitochondrial proton leak.
[a]$P < 0.01$ level (two-tailed).
[b]$P < 0.05$ level (two-tailed).

likely capture related but distinct aspects of mitochondrial biology. This distinction provides important context for the neurocognitive findings, as mtDNAcn and mitochondrial function demonstrated different patterns of association with cognitive outcomes, suggesting that these metrics provide distinct, rather than redundant, information regarding the relationship between mitochondrial health and cognition in WWH. These findings underscore the importance of examining multiple mitochondrial markers when evaluating mitochondrial contributions to cognitive impairment in HIV. This work also provides a foundation for future longitudinal and investigational studies investigating mitochondrial modifications and whether or not improvement in mitochondrial health is associated with improvements in cognitive outcomes, particularly memory.

Several limitations should be considered when interpreting these findings. First, this study focused on WWH enriched for cognitive impairment, elevated stress, and mental health disorders, which may limit generalizability to other populations. For example, previous work has demonstrated sex differences in mitochondrial health, especially related to mtDNAcn [46]; as the current study does not include men with HIV, future work is needed to understand how the relationships between mitochondrial health and cognitive outcomes differ in men with HIV. That said, the current study provides insight into mitochondrial dynamics within a particularly vulnerable subset of women living with both HIV and psychiatric comorbidities at high risk for cognitive impairment. Second, the study was cross-sectional, limiting the ability to determine temporal or causal relationships between mitochondrial markers and cognitive outcomes, and instead represents preliminary analysis of these important biological outcomes. Given the dynamic nature of mitochondrial biology, longitudinal studies are needed to determine how mitochondrial measures relate to cognitive trajectories over time. Third, mitochondrial analyses were conducted in PBMCs, which represent a heterogeneous mixture of immune cells with distinct mitochondrial properties. Future work examining cell-type–specific mitochondrial function may provide additional insight into these relationships. Additionally, mitochondrial function is highly tissue-specific, and mitochondrial measures obtained from PBMCs should not be interpreted as directly reflecting mitochondrial function within the CNS. Future work is needed to further examine biological mechanisms explaining the relationship observed here between peripheral mitochondrial function and cognitive outcomes. Finally, although proton leak may reflect oxidative stress-related mitochondrial damage, direct measures of oxidative stress were not included in this study. Integrating mitochondrial bioenergetic measures with markers of oxidative stress and other mitochondrial pathways may help clarify possible mechanisms linking peripheral mitochondrial dysfunction and cognition.

Overall, these findings demonstrate that mitochondrial genomic content and mitochondrial bioenergetic function capture overlapping but nonredundant aspects of mitochondrial biology in WWH. Mitochondrial proton leak emerged as the mitochondrial marker most strongly associated with memory performance, suggesting that markers of mitochondrial dysfunction rather than mitochondrial abundance alone may be more closely related to cognitive outcomes in this population. These results provide initial evidence linking peripheral

mitochondrial dysfunction with memory performance in WWH and highlight mitochondrial bioenergetic pathways as an important area for future investigation.

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Conflicts of interest

There are no conflicts of interest.

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