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Mitochondrial Dysfunction and Insulin Resistance in Pubertal Youth Living with Perinatally Acquired HIV

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Abstract

Mitochondrial dysfunction (MD) is linked to cardiometabolic complications, such as obesity and insulin resistance (IR), the frequencies of which are higher in adults living with HIV infection and receiving combination antiretroviral therapies (ARV). ARV-treated youth living with perinatally acquired HIV infection (YLPHIV) may be especially susceptible to IR due to long-term exposure to both factors. Medical histories, fasting blood chemistry panels, and mitochondrial function in banked peripheral blood mononuclear cells (PBMCs) were assessed in eligible YLPHIV from the Pediatric HIV/AIDS Cohort Study (PHACS)/Adolescent Master Protocol (AMP) Mitochondrial Determinants Component cohort, stratified by Homeostatic Model Assessment of IR (HOMA-IR) score: case (score ≥4, $n = 39$) or control (score <4, $n = 105$). PBMCs were sources for mitochondrial (mt) DNA copies/cell; mtRNA transcript levels of oxidative phosphorylation (OXPHOS) subunits NADH dehydrogenases 1 and 6, and cytochrome B; and enzymatic activities of OXPHOS Complexes I (CI) and IV (CIV). Logistic regression models were fit to estimate the odds of IR case diagnosis, adjusted for sex, race/ethnicity, body mass index (BMI) z-score, and Tanner stage. IR cases were similar to controls by age, sex, and race/ethnicity. Cases had higher median levels of peak HIV viral load, lactate, pyruvate, triglycerides, and BMI z-scores. OXPHOS CI enzymatic activity was lower in cases ($\log_{10}$ 1.62 vs. 1.70) and inversely correlated with HOMA-IR score ($r = -0.157$, $p = .061$), but did not associate with IR in adjusted models. Fully adjusted models indicated associations of nadir CD4% [odds ratio (OR) = 0.95, 95% confidence intervals (CIs) = 0.90–1.00] or peak HIV load (OR = 3.48, 95% CIs = 1.70–10.79) with IR. IR in YLPHIV was strongly associated with morphometrics, but early virologic and immunologic factors may also influence MD.

Keywords: adolescent, HIV, insulin resistance, mitochondria, oxidative phosphorylation, type 2 diabetes

Introduction

Combination antiretroviral therapy (cART) has improved morbidity and mortality rates for individuals with HIV infection, but is associated with an increase in long-term cardiometabolic complications such as insulin resistance (IR) and type 2 diabetes (T2Ds).[1,2] Mitochondrial dysfunction (MD) is purported to be one of the factors that underlies these complications and has been linked to HIV infection[3–8] and/or related cART regimens.[9–13] Children who are exposed to both factors perinatally may therefore be particularly vulnerable to developing MD and pursuant cardiometabolic comorbidities.

Multiple recent reports have described the effects of HIV infection upon MD in immune cells and the resulting shifts in bioenergetic capacities of the surviving peripheral blood mononuclear cells (PBMCs).[14–17] Early HIV infectivity is selective for CD4+ T cells with high levels of mitochondrial oxidative phosphorylation (OXPHOS),[18] and chronic infection may result due to alterations in immune subset-specific differentiation potentials driven by modulation of OXPHOS/glycolysis usage and concurrent transcriptional

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programming.[19,20] Intriguingly, individuals considered that ''elite HIV controllers'' may successfully respond to HIV infection due to their possession of CD8+ T cell subsets displaying highly plastic metabolism, such as greater capacities for both OXPHOS and glycolysis, compared to the primarily glycolytic dependency of CD8+ T cells in noncontrollers.[21] OXPHOS capacities of CD4+ T cells from HIV-uninfected adults are negatively impacted by exposure to certain cART drugs, specifically newer integrase strand transfer inhibitors; and CD4+ T cells from cART-naive adults living with HIV displayed impaired basal and maximal OXPHOS levels.[22] Thus, both HIV infection itself and cART may shape composition and bioenergetics of surviving PBMC via induction of MD, potentially increasing the likelihood of cardiometabolic abnormalities.

We have shown in the Adolescent Master Protocol (AMP) of the Pediatric HIV/AIDS Cohort Study (PHACS) network that youth living with perinatally acquired HIV (YLPHIV) have elevated biomarkers for cardiometabolic dysfunction at early ages,[23] as well as elevated prevalence of IR compared with that reported in HIV-uninfected nonobese youth.[24] Data from a pilot study of PHACS AMP participants suggested that mtDNA copies/cell were lower in PBMCs of YLPHIV with IR compared to those with normal insulin sensitivity, and that fasting glucose level was inversely proportional to OXPHOS Complex I (CI) enzymatic activity.[25] In this population, IR was associated with obesity, higher CD4+ T cell percentages at nadir, and amprenavir usage.[26] Another recent pilot study of YLPHIV described lower rates of basal and maximal OXPHOS in PBMCs from those with IR, which was associated with higher body mass index (BMI) z-score and venous metabolites: pyruvate (Pyr), lactate (Lac), and triglycerides.[27] In adults living with HIV, reductions in PBMC OXPHOS activities are correlated with lower levels found in adipose tissue,[28] and a recent study indicates that activity levels of OXPHOS CI and Complex IV (CIV) in adipose tissue inversely correlated with IR,[29] suggesting that detectable changes in PBMC levels may be indicative of systemic MD.

Due to our observations of lowered OXPHOS capacities being associated with IR in YLPHIV, we sought to further assess the relationship of MD and IR by examining a broader range of mitochondrial measures in PBMC from a larger population of YLPHIV. We hypothesized that levels of mtDNA per cell, mtRNA OXPHOS-related transcripts, and OXPHOS subunit enzymatic activity within PBMC would inversely correlate with Homeostatic Model Assessment of IR (HOMA-IR) scores, a widely used measure of insulin sensitivity in epidemiologic, population-based, and other group-level assessments.[30]

Materials and Methods

Study design and population

The study population was enrolled from a cohort of YLPHIV in the Mitochondrial Determinants Component (MDC), a substudy of the multicenter U.S.-based AMP study (PHACS network; https://phacsstudy.org/About-Us/Active-Protocols), which is an ongoing, prospective study evaluating long-term effects of HIV infection and cART in YLPHIV (aged 7 to <16 years at entry; $n = 451$). Of these 451 participants, 243 were enrolled in MDC; children were excluded from enrollment if they had any known mitochondrial abnormalities, Type I diabetes, liver dysfunction (including hepatitis B or C), or other conditions known to alter mitochondrial function or Lac levels. For the analyses herein, we used previously established HOMA-IR cutoffs[31] to assess a subset of 39 insulin-resistant (IR+) cases (baseline HOMA-IR score >4) and 105 randomly selected insulin-sensitive (IR–) controls (HOMA-IR score <4), both at Tanner stage >1; participants were included if sufficient aliquots of PBMC were available for assays. HOMA-IR score was calculated as described previously[32]: ([fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5). The AMP protocol was approved by the Institutional Review Board (IRB) at each participating site and by that of the Harvard T.H. Chan School of Public Health. Written informed consent was obtained from each youth's parent or legal guardian, and assent was obtained from youth participants according to local IRB guidelines.

Clinical and laboratory measurements

Routine clinical measurements for both cases and controls included anthropometric measures [height, weight, BMI] and fasting blood measures of glucose, insulin, lipids, Lac, and Pyr following PHACS AMP Protocol version 4.0, based on the ACTG/IMPAACT Laboratory Manual, available at: https://www.hanc.info/labs/labresources/procedures/Pages/actgImpaactLabManual.aspx. In brief, glucose and insulin measurements were performed at the Diabetes Research Institute Clinical Chemistry Laboratory at the University of Miami on a Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN) following manufacturer's specifications. Lipids, Lac, and Pyr levels were determined at each clinical site following above-mentioned, standardized protocols. Abnormal Lac was defined as >2.0 mmol/L. If Lac was >2.0 mmol/L, but the Lac/Pyr ratio was ≤20, a secondary collection was performed to determine Lac and Pyr concentrations.

Absolute CD4+ T cell (CD4) count and percentages, plasma HIV-1 RNA (viral load), and antiretroviral (ARV) regimen history were abstracted from medical chart histories. Use of individual ARV were assessed based on whether patients were currently receiving the medication, had ever received the medication, and cumulative duration of medication usage.

Mitochondrial assays

PBMCs were obtained using EDTA vacutainers within 8 h of phlebotomy and processed according to AIDS Clinical Trials Group protocols (https://www.hanc.info/labs/labresources/procedures/Pages/actgImpaactLabManual.aspx). PBMCs were isolated using the Ficoll-Hypaque Overlay Method and cells (5.0 × 10^6 cells/0.5 mL) were cryopreserved (−140°C) and shipped quarterly to the University of Hawaii on dry ice.

PBMCs mtRNA and mtDNA levels were calculated as done previously using relative and absolute quantitative PCR, respectively.[33] In brief, transcript levels of nuclear-encoded, mitochondrial ribosomal protein *L13* RNA were used for normalization of mitochondrial-encoded genes associated with OXPHOS: cytochrome b (*CYTB*) and NADH dehydrogenase (ND) subunits 1 (*ND1*) and 6 (*ND6*). Transcript levels were calculated as ratios of each of the OXPHOS mtRNA targets cycle threshold to the cycle threshold value of *L13* mRNA, and all samples were run in duplicate. Copy number of mtDNA per cell was assayed by comparing qPCR

amplification thresholds of the mitochondrial DNA target, ND subunit 2, to those of the nuclear target, Fas Ligand, and across each sample; all samples and standards were run in duplicate. A control DNA plasmid containing both target regions was serially diluted and used to generate a standard curve of cycle thresholds, against which absolute values were derived for samples. Results were analyzed with Version 1.5.0 LightCycler 480 software.[25]

Measurement of OXPHOS CI and CIV enzymatic activities was performed in duplicate by thin-layer chromatography and immunoassays as described previously.[25] Activity was measured as optical density/μg of protein $\times 10^3$.

Data analyses

Characteristics of cases and controls, including sociodemographic data, metabolic measures, and HIV-specific characteristics, were summarized by the frequency for categorical variables or median [interquartile ranges (IQRs)] for continuous variables, and comparisons between the two groups were performed in using Fisher's exact test for categorical variables and Wilcoxon Rank Sum test for continuous variables. Mitochondrial measures (mtDNA, *ND1/L13*, *ND6/L13*, *CYTB/L13*, CI activity, and CIV activity) were $\log_{10}$ transformed before analysis. Associations between the mitochondrial and clinical measures were evaluated by Spearman correlations. Logistic regression models were fit to evaluate the odds of being a case compared to a control with each one unit increase in each mitochondrial measure, CD4 value, and viral load, unadjusted and adjusted for sex, race/ethnicity, BMI z-score, and Tanner stage group (2–3 vs. 4–5). We report herein odds ratios (ORs) and 95% confidence intervals (CIs) for variables with *p*-values below .10.

Results

Demographic and clinical characteristics of IR+ cases ($n = 39$) and IR− controls ($n = 105$) are presented in Tables 1 and 2. There were no differences observed between cases and controls by age, sex, race/ethnicity, Tanner stage, current CD4 values, or current HIV viral load (Table 1). Cases demonstrated a higher peak $\log_{10}$ HIV viral RNA load (median 5.78 vs. 5.50, $p = .002$), and lower values for CD4 at nadir compared to controls. Median values of the body

Table 1. Characteristics of Participants by Insulin Resistance Status

Characteristic	*N (IR+/IR−)*	*IR+*	*IR−*	p[a]
Age (year), median [IQR]	39/105	16.37 [14.52, 17.99]	16.08 [13.99, 17.65]	.30
Sex [Female, *n* (%]	39/105	22 (56)	53 (50)	.58
Race/Ethnicity, *n* (%)	39/105			.62
Hispanic		10 (26)	25 (33)	
Black non-Hispanic		27 (69)	66 (63)	
White/other non-Hispanic		2 (5)	4 (4)	
Tanner stage, *n* (%)	39/105			.12
2–3		5 (13)	28 (27)	
4–5		34 (87)	77 (73)	
Current CD4 (cells/mm^3), median [IQR]	38/94	563.50 [426.00, 864.00]	620.50 [461.00, 777.00]	.90
Current CD4 (cells/mm^3), *n* (%)	38/94			.23
<200		5 (13)	5 (5)	
201–500		7 (18)	25 (24)	
>500		26 (67)	64 (61)	
Current CD4 (%), median [IQR]	38/94	31.35 [25.00, 36.00]	32.40 [25.10, 38.00]	.41
Nadir CD4 (cells/mm^3), median [IQR]	39/105	268 [70, 443]	328 [204, 454]	.068
Nadir CD4 (cells/mm^3), *n* (%)	39/105			.090
<200		16 (41)	24 (23)	
201–500		18 (46)	60 (57)	
≥500		5 (13)	21 (20)	
Nadir CD4 (%), median [IQR]	39/105	15.00 [4.80, 21.70]	17.15 [10.00, 24.00]	.058
Current log HIV RNA (copies/mL), median [IQR]	35/96	1.73 [1.60, 3.13]	2.30 [1.60, 3.53]	.49
Peak log HIV RNA (copies/mL), median [IQR]	39/105	5.78 [5.49, 5.88]	5.50 [4.95, 5.87]	.002
Cumulative duration of ARVs, (years), median [IQR]	39/105			
ZDV		3.47 [0.92, 6.49]	3.31 [0.61, 7.85]	.77
D4T		5.22 [1.46, 7.13]	5.64 [0.00, 8.62]	.83
3TC		7.21 [3.98, 10.66]	6.32 [3.29, 9.89]	.36
TDF		1.38 [0.00, 3.56]	0.00 [0.00, 1.83]	.061
BMI z-score, median [IQR]	38/105	1.12 [0.57, 1.70]	0.09 [−0.57, 0.73]	<.001
Waist-to-hip ratio, median [IQR]	35/101	0.90 [0.87, 0.97]	0.86 [0.83, 0.91]	.002

[a]Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables.

3TC, lamivudine; ARV, antiretrovirals; BMI, body mass index; D4T, stavudine; IQR, interquartile range; IR+, insulin-resistant case; IR−, insulin-sensitive control; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

Table 2. Metabolic Characteristics by Insulin Resistance Status

Characteristic	*N (IR+/IR−)*	*IR+*	*IR−*	p[a]
Venous Lac (mmol/L), median [IQR]	37/103	1.25 [0.80, 1.70]	1.04 [0.78, 1.22]	.021
Venous Pyr (mmol/L), median [IQR]	35/102	0.10 [0.07, 0.15]	0.08 [0.02, 0.11]	.015
Lac to Pyr ratios, median [IQR]	34/103	11.43 [9.22, 17.33]	14.04 [9.60, 32.25]	.36
Fasting LDL, median [IQR] (mg/dL)	38/101	89.50 [69.00, 123.00]	85.00 [66.00, 106.00]	.088
Fasting HDL, median [IQR]	38/101	44.50 [41.00, 56.00]	48.00 [41.00, 58.00]	.47
Fasting triglycerides, median [IQR] (mg/dL)	38/102	105.50 [82.00, 139.00]	81.00 [58.00, 103.00]	<.001
Fasting total cholesterol, median [IQR] (mg/dL)	38/102	165.50 [142.00, 193.00]	150.50 [129.00, 179.00]	.035
Fasting glucose (mg/dL)	39/105	89.00 [84.00, 93.00]	84.00 [81.00, 89.00]	.002
Fasting insulin (pU/mL)	39/105	25.20 [22.40, 33.50]	10.90 [8.00, 14.50]	<.001
HOMA-IR	39/105	5.55 [4.77, 7.69]	2.36 [1.58, 304]	<.001

[a]Wilcoxon Rank Sum test.
HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of insulin resistance; Lac, lactate; LDL, low-density lipoprotein; Pyr, pyruvate.

composition measures BMI z-score (1.12 vs. 0.09, $p<.001$) and waist-to-hip ratio (0.90 vs. 0.86, $p=.002$) were higher in cases than controls. The groups were similar on current use and duration of ARVs with the exception of greater cumulative duration of tenofovir disoproxil fumarate (TDF) use in IR+ cases (median of 1.38 vs. 0.00 years, $p=.061$). Median levels of Lac, Pyr, low-density lipoprotein (LDL), triglycerides, cholesterol, glucose, and insulin were higher in IR+ cases than controls (Table 2).

In comparing the mitochondrial measures between cases and controls (Table 3), there were no significant differences in mtDNA copies/cell or any of the selected OXPHOS-related mtRNA levels. However, enzymatic OXPHOS activity levels of CI were lower in IR+ cases compared to IR− controls, but no difference was noted in CIV enzyme activity

Across all samples, positive Spearman correlation coefficients were observed between levels of Lac and $\log_{10}$-transformed values of the following: mtDNA ($r=0.18$, $p=.037$), *CYTB* ($r=0.22$, $p=.009$), *ND6* ($r=0.23$, $p=.008$), and *ND1* ($r=0.25$, $p=.004$). Lac/Pyr ratios positively correlated with $\log_{10}$ mtDNA copies/cell ($r=0.23$, $p=.007$), $\log_{10}$ *ND1/L13* ($r=0.20$, $p=.018$), and weakly with $\log_{10}$ *ND6/L13* ($r=0.16$, $p=.070$). HOMA-IR score was significantly correlated with waist-to-hip ratio ($r=0.37$, $p<.001$) and BMI z-score ($r=0.47$, $p<.001$). HOMA-IR also positively associated with fasting triglycerides ($r=0.31$, $p<.001$), and weakly with fasting total cholesterol ($r=0.16$, $p=.06$), and LDL ($r=0.15$, $p=.069$). There was a weak inverse correlation of HOMA-IR score with $\log_{10}$ CI activity ($r=-0.16$, $p=.061$), but a significant positive correlation with peak HIV RNA load ($r=0.24$, $p=.003$).

Further associations of early immunological and virological indicators with MD measures are shown in Table 4. In cases and controls combined, levels of both mtDNA copies/cell and *ND1/L13* negatively correlated with both nadir CD4 count and percentage (Table 4). No associations between current and peak HIV viral loads were observed with any of the mitochondrial measures. Current CD4 counts did not correlate with mitochondrial measures, but current CD4 percentage was negatively associated with $\log_{10}$ *ND6/L13* ($r=-0.18$, $p=.037$) and positively associated with $\log_{10}$ CI activity ($r=0.17$, $p=.048$). Yet, no correlations were observed between activity levels of either of the OXPHOS complexes with mtRNA or mtDNA levels.

Univariable logistic regression analyses noted no effects of specific ARV usage, age, sex, or race/ethnicity and were not associated with IR status; however, there was 2.47 times higher odds of IR (95% CIs = 0.88–6.95, $p=.086$) for Tanner stage 4–5 group versus 2–3 group, and 2.66 times higher odds of IR (95% CIs = 1.69–4.19, $p<.001$) with each one unit increase in BMI z-score. With each one unit increase in *ND6/L13* ratio, the OR of being IR+ versus IR− was 1.22 times higher (95% CIs = 0.96–1.54, $p=.099$), and for $\log_{10}$ CI activity it was 0.16 times lower (95% CIs = 0.02–1.31, $p=.087$), both before adjusting models for sex, race/ethnicity, BMI z-score, and Tanner stage. When $\log_{10}$ mtDNA copies/cell count was divided into quartiles, the odds of being a case was 0.20 times lower in quartile 2 compared with quartile 1 (95% CIs = 0.05–0.79; $p=.022$), before full adjustment. All these relationships persisted after full adjustment, but *p*-values were above $p=.10$ and thus did not reach threshold for reporting, nor did other mitochondrial markers assessed herein. Current

Table 3. Mitochondrial Characteristics by Insulin Resistance Status

Characteristic	*N (IR+/IR−)*	*IR+*	*IR−*	p[a]
Log_{10} mtDNA (copies/cell), median [IQR]	39/105	2.16 [1.98, 2.27]	2.11 [2.01, 2.24]	.67
Log_{10} *ND1/L13*, median [IQR]	36/105	0.64 [0.56, 0.79]	0.66 [0.52, 0.87]	.98
Log_{10} *ND6/L13*, median [IQR]	36/105	0.25 [0.15, 0.40]	0.24 [0.12, 0.41]	.66
Log_{10} *CYTB/L13*, median [IQR]	36/105	0.57 [0.46, 0.71]	0.60 [0.46, 0.78]	.86
Log_{10} CI activity, median [IQR]	39/104	1.62 [1.48, 1.72]	1.70 [1.55, 1.79]	.057
Log_{10} CIV activity, median [IQR]	39/104	1.90 [1.60, 2.09]	1.88 [1.76, 1.94]	.32

[a]Wilcoxon Rank Sum test.
CI, Complex I; CIV, Complex IV; *CYTB*, cytochrome B; *L13*, large ribosomal subunit 13; mtDNA, mitochondrial DNA; ND, NADH dehydrogenase.

Table 4. Spearman Correlations of Nadir CD4 Values or Peak HIV RNA Load with Markers of Mitochondrial Function

	Nadir CD4 count		*Nadir CD4 percent*		*Peak log HIV RNA load*	
	ρ	p	*ρ*	p	*ρ*	p
Log_{10} mtDNA copies	–0.234	.005	–0.261	.002	0.027	.75
Log_{10} *ND1/L13*	–0.167	.048	–0.159	.060	0.058	.50
Log_{10} *ND6/L13*	–0.129	.13	–0.104	.22	–0.028	.73
Log_{10} *CYTB/L13*	–0.092	.28	–0.056	.51	0.006	.95
Log_{10} CI activity	0.044	.58	0.061	.47	0.042	.62
Log_{10} CIV activity	0.073	.38	0.114	.18	0.018	.83

values for CD4 and HIV viral load were not asFsociated with a higher OR of IR before or after adjustment, but nadir CD4 count/percentage and peak HIV RNA load were associated with lower and higher odds of IR, respectively, both in unadjusted and adjusted models (Table 5).

Discussion

Our study lends further support to past observations suggesting traditional morphometric measures, that is, BMI z-scores and waist-to-hip ratios, and venous Lac and Pyr concentrations as indicators of IR incidence. We interpret our data to suggest that there may be links between initial immunovirologic responses, current mitochondrial function, and IR in youth living with perinatally acquired HIV, beyond the standard associations of body composition and metabolite presentations. Specifically, we observed odds risk elevation for IR with higher mitochondrial RNA transcripts, with lower OXPHOS enzymatic activity, and with lower mitochondrial DNA copy numbers. Furthermore, measures of mitochondrial RNA and DNA correlated with nadir CD4 values, which themselves impacted odds risk for IR case diagnosis. Finally, peak HIV RNA load was itself linked to elevated odds risk for IR case diagnosis. Taken together, we suggest that earlier interventions may assist in alleviating cardiometabolic complications.

Understanding how obesity, IR, T2D, and cardiovascular disease progression evolves is still poorly understood in YLPHIV. This cardiometabolic cascade has, however, been consistently linked to inflammatory processes in the general population,[34,35] as well as in adults living with HIV.[36,37] The influence of immune cell cross talk with adipocytes is known to impact obesity and metabolic dysfunction,[38–42] with increasing evidence that immune cell mitochondrial function determines both the inflammatory properties[14,17,19] and the composition of surviving PBMC subsets in adults living with HIV.[20,21,43] Recent studies by our group, of adults living with HIV, highlight that there is a positive correlation between PBMC levels of OXPHOS subunit proteins with those in adipose tissue[28]; that intracellular ATP levels strongly correlate between the adipocyte and the immune cell-containing preadipocyte fractions of subcutaneous fat, that these ATP levels change after initial HIV infection and after development of lipoatrophy[33]; and that activity levels of OXPHOS CI and CIV in adipose tissue inversely correlate with HOMA-IR score.[29] Thus, changes in the metabolic function of PBMCs, such as those measured here, may act as systemic indicators of MD and inflammation, which are involved in adipose tissue homeostasis.

In our study, a trend toward lower OXPHOS CI activity was found in PBMCs of YLPHIV displaying IR, and IR moderately associated with lower CI activity in logistic regression models before full adjustment. Intriguingly, CI activity weakly, but positively, correlated with current CD4 percentage, while *ND6* mtRNA levels inversely associated with current CD4 percentage. Slight associations of *ND6* mtRNA measures and quartile ranges of mtDNA with IR risk were observed in regression models, but lost significance after full adjustment. Combined, these observations may lend support to the hypothesis of a compensatory response by CI activity to higher sustained insulin levels put forth previously.[25] However, a more likely alternative is that these observations simply reflect the compositional changes that activated and/or Glut1+ CD4+ T cell subsets comprise in total PBMCs,[44] indicative of the glycolytic shifts observed following HIV infection[18] and in HIV noncontrollers.[21] Thus, possessing OXPHOS-centric CD4+ T cells may relate to euglycemia after HIV infection.

Nadir CD4 values inversely correlated with mtDNA and *ND1* mtRNA levels in our study, and had significant, although minimal, associations with IR status. One recent study of the AMP cohort found an increased mean incidence of IR compared to a past study of AMP participants, with no associations found with CD4 or viral load measures, but there was concern for drawing comparisons due to confounding effects of Tanner stage upon metabolism.[24] A separate study of AMP participants also found associations of nadir CD4 percentages with IR,[26] although in an opposite direction to our observation, thus highlighting the need for longitudinal assessments. Interestingly, a loss of CD4+ T cells that home

Table 5. Unadjusted and Adjusted Odds Ratios Assessing the Associations of Nadir CD4 Values or Peak HIV RNA Load with Insulin Resistance Status (N = 143)

	Unadjusted			*Adjusted*[a]		
Predictor of interest	*OR*	*95% CIs*	p	*OR*	*95% CIs*	p
Nadir CD4 (100 cells/mm^3)	0.84	0.70–1.00	.056	0.81	0.65–1.00	.055
Nadir CD4 percent (%)	0.96	0.92–1.00	.058	0.95	0.90–1.00	.043
Peak log HIV RNA (copies/mL)	3.45	1.53–7.78	.003	4.28	1.70–10.79	.002

[a]All models adjusted for sex, race/ethnicity, BMI z-score, and Tanner stage.
CIs, confidence intervals; OR, odds ratio.

to the gut and provide protection against microbial translocation has been shown in those living with HIV,[45] and low nadir CD4 values in youth and adults living with HIV associate with higher levels of gut dysbiosis,[46,47] which is further linked to circulating inflammatory markers,[48,49] obesity, IR, and T2D.[50–52] Ugandan YLPHIV showed higher incidence rates of IR while on certain therapy regimens after 48 weeks, and HOMA-IR score associated with soluble CD163,[53] which itself has been shown to be released by adipose tissue in response to microbial products[54] and to be related to insulin sensitivity.[55,56] Together, these studies indicate that maintenance of immune cells responsible for controlling both HIV and microbial infections may be linked to IR incidence.

HIV viral load is associated with greater endothelial dysfunction and inflammatory markers in PHACS participants,[23] with the extent of immunological and microbiological dysbiosis in the gut of adults living with HIV,[57,58] and, in general, has been a good indicator of disease progression.[59,60] Our data showed no difference in current HIV RNA loads; however, peak detected values, taken from medical histories, were significantly linked with IR in both unadjusted and adjusted models. Viral load has been further associated with metabolic syndrome,[61] hypertriglyceridemia,[62] and glycosylated hemoglobin A1c level,[63] an indicator of blood glucose control; however, not all studies show a relationship between viral load and HOMA scores,[64] indicating that future studies are needed in this arena.

A notable strength of our study was the ability to include data from a large number of YLPHIV from multiple centers. Although IR is prevalent in our population, very few YLPHIV have developed frank T2D and, therefore, the ability to evaluate MD in this setting has been limited. Due to the cross-sectional nature of our study here, directionality of associations is uncertain. Longitudinal follow-up of the participants in this cohort with serial evaluation of mitochondrial measures will reveal the relationships between trajectories of such changes and the progression to T2D. These studies will also help to correct for the effects of Tanner stage, which may have had some contribution here, but were adjusted for in our models. Future longitudinal studies would also benefit by closely monitoring immunologic responses to both HIV and microbial translocation to assess gut dysbiosis, with prophylactic prevention of inflammation via antibiotic administration becoming a thought-provoking intervention strategy in those at higher risk.[65] Finally, ARV usages and durations did not have an effect on IR and MD in YLPHIV. There was a trend of increased duration of TDF usage in the IR+ compared to IR–. TDF has been associated with renal or adipose MD in adults living with HIV.[66–68] Future studies are needed to evaluate TDF in YLPHIV and IR.

While our hypothesis that measures associated with MD would be different in PBMCs from YLPHIV displaying IR was not fully corroborated, novel observations in this study add to our understanding of IR progression in these youth at high risk of cardiometabolic dysfunction. Specifically, we observed trends in PBMC CI activity and nadir CD4 values to be inversely associated with IR; and for venous metabolite, *ND6* mtRNA, and peak HIV RNA levels to be positively associated with IR in YLPHIV. Furthermore, Lac levels significantly correlated to mtDNA and mtRNA copy numbers, while current CD4 percentage inversely associated with *ND6* mtRNA, but positively with CI activity. Together, we interpret these data to suggest that IR in YLPHIV is linked to mitochondrial, immunologic, and virologic factors, and warrant further studies to assess importance of changes in mitochondrial function in PBMCs as predictors for IR and T2D susceptibility.

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Disclaimer

The conclusions and opinions expressed in this article are those of the authors and do not necessarily reflect those of the National Institutes of Health or U.S. Department of Health and Human Services.

Author Disclosure Statement

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References

1. Capeau J, Bouteloup V, Katlama C, *et al.*: Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. AIDS 2012;26:303–314.
2. Fiseha T, Belete AG: Diabetes mellitus and its associated factors among human immunodeficiency virus-infected patients on anti-retroviral therapy in Northeast Ethiopia. BMC Res Notes 2019;12:372.
3. Cheney L, Hou JC, Morrison S, Pessin J, Steigbigel RT: Nef inhibits glucose uptake in adipocytes and contributes to insulin resistance in human immunodeficiency virus type I infection. J Infect Dis 2011;203:1824–1831.
4. Lecoeur H, Borgne-Sanchez A, Chaloin O, *et al.*: HIV-1 Tat protein directly induces mitochondrial membrane permeabilization and inactivates cytochrome *c* oxidase. Cell Death Dis 2012;3:e282.
5. Ogawa M, Takemoto Y, Sumi S, *et al.*: ATP generation in a host cell in early-phase infection is increased by upregulation of cytochrome c oxidase activity via the p2 peptide from human immunodeficiency virus type 1 Gag. Retrovirology 2015;12:97.
6. Rodríguez-Mora S, Mateos E, Moran M, *et al.*: Intracellular expression of Tat alters mitochondrial functions in T cells: A potential mechanism to understand mitochondrial damage during HIV-1 replication. Retrovirology 2015;12:78.
7. Villeneuve LM, Purnell PR, Stauch KL, Callen SE, Buch SJ, Fox HS: HIV-1 transgenic rats display mitochondrial abnormalities consistent with abnormal energy generation and distribution. J Neurovirol 2016;22:564–574.
8. Sun J, Longchamps RJ, Piggott DA, *et al.*: The association between HIV infection and mitochondrial DNA copy number in peripheral blood: A population-based, prospective cohort study. J Infect Dis 2019;219:1285–1293.
9. Dagan T, Sable C, Bray J, Gerschenson M: Mitochondrial dysfunction and antiretroviral nucleoside analog toxicities: What is the evidence? Mitochondrion 2002;1:397–412.
10. Poirier MC, Divi RL, Al-Harthi L, *et al.*: Long-term mitochondrial toxicity in HIV-uninfected infants born to HIV-infected mothers. J Acquir Immune Defic Syndr 2003;33: 175–183.
11. McComsey GA, Libutti DE, O'Riordan M, *et al.*: Mitochondrial RNA and DNA alterations in HIV lipoatrophy are linked to antiretroviral therapy and not to HIV infection. Antivir Ther (Lond) 2008;13:715–722.
12. Crain MJ, Chernoff MC, Oleske JM, *et al.*: Possible mitochondrial dysfunction and its association with antiretroviral therapy use in children perinatally infected with HIV. J Infect Dis 2010;202:291–301.
13. Sims EK, Park G, Mather KJ, Mirmira RG, Liu Z, Gupta SK: Immune reconstitution in ART treated, but not untreated HIV infection, is associated with abnormal beta cell function. PLoS One 2018;13:e0197080.
14. Yu F, Hao Y, Zhao H, *et al.*: Distinct mitochondrial disturbance in CD4+T and CD8+T cells from HIV-infected patients. J Acquir Immune Defic Syndr 2017;74:206–212.
15. Klein Geltink RI: The metabolic tug of war between HIV and T cells. Nat Metab 2019;1:653–655.
16. Gubser C, Pitman MC, Lewin SR: CD4+ T cell signatures in HIV infection. Nat Immunol 2019;20:948–950.
17. Castellano P, Prevedel L, Valdebenito S, Eugenin EA: HIV infection and latency induce a unique metabolic signature in human macrophages. Sci Rep 2019;9:1–14.
18. Valle-Casuso JC, Angin M, Volant S, *et al.*: Cellular metabolism is a major determinant of HIV-1 reservoir seeding in CD4+ T cells and offers an opportunity to tackle infection. Cell Metab 2019;29:611–626.e5.
19. Bailis W, Shyer JA, Zhao J, *et al.*: Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. Nature 2019;571:403–407.
20. Morou A, Brunet-Ratnasingham E, Dubé M, *et al.*: Altered differentiation is central to HIV-specific CD4 + T cell dysfunction in progressive disease. Nat Immunol 2019;20: 1059–1070.
21. Angin M, Volant S, Passaes C, *et al.*: Metabolic plasticity of HIV-specific CD8 + T cells is associated with enhanced antiviral potential and natural control of HIV-1 infection. Nat Metab 2019;1:704–716.
22. Korencak M, Byrne M, Richter E, *et al.*: Effect of HIV infection and antiretroviral therapy on immune cellular functions. JCI Insight 2019;4:e126675.
23. Miller TI, Borkowsky W, DiMeglio LA, *et al.*: Metabolic abnormalities and viral replication are associated with biomarkers of vascular dysfunction in HIV-infected children. HIV Med 2012;13:264–275.
24. Geffner ME, Patel K, Jacobson DL, *et al.*: Changes in insulin sensitivity over time and associated factors in HIV-infected adolescents. AIDS 2018;32:613–622.
25. Sharma TS, Jacobson DL, Anderson L, *et al.*: Short communication: The relationship between mitochondrial dysfunction and insulin eesistance in HIV-infected children receiving antiretroviral therapy. AIDS Res Hum Retroviruses 2013;29:1211–1217.
26. Geffner ME, Patel K, Miller TL, *et al.*: Factors associated with insulin resistance among children and adolescents perinatally infected with HIV-1 in the pediatric HIV/AIDS cohort study. Horm Res Paediatr 2011;76:386–391.
27. Takemoto JK, Miller TL, Wang J, *et al.*: Insulin resistance in HIV-infected youth is associated with decreased mitochondrial respiration. AIDS 2017;31:15–23.
28. Shikuma CM, Gerschenson M, Chow D, *et al.*: Mitochondrial oxidative phosphorylation protein levels in peripheral blood mononuclear cells correlate with levels in subcutaneous adipose tissue within samples differing by HIV and lipoatrophy status. AIDS Res Hum Retroviruses 2008;24:1255–1262.

29. Hulgan T, Ramsey BS, Koethe JR, *et al.*: Relationships between adipose mitochondrial function, serum adiponectin, and insulin resistance in persons with HIV after 96 weeks of antiretroviral therapy. J Acquir Immune Defic Syndr 2019;80:358–366.
30. Tagi VM, Giannini C, Chiarelli F: Insulin resistance in children. Front Endocrinol (Lausanne) 2019;10:342.
31. Valerio G, Licenziati MR, Iannuzzi A, *et al.*: Insulin resistance and impaired glucose tolerance in obese children and adolescents from Southern Italy. Nutr Metab Cardiovasc Dis 2006;16:279–284.
32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419.
33. Gojanovich GS, Shikuma C, Milne C, LiButti DE, Chow D, Gerschenson M: Subcutaneous adipocyte adenosine triphosphate levels in HIV infected patients. AIDS Res Hum Retroviruses 2020;36:75–82.
34. Romeo GR, Lee J, Shoelson SE: Metabolic syndrome, insulin resistance, and roles of inflammation—Mechanisms and therapeutic targets. Arterioscler Thromb Vasc Biol 2012;32:1771–1776.
35. Saltiel AR, Olefsky JM: Inflammatory mechanisms linking obesity and metabolic disease. J Clin Invest 2017;127:1–4.
36. Psomas C, Younas M, Reynes C, *et al.*: One of the immune activation profiles observed in HIV-1-infected adults with suppressed viremia is linked to metabolic syndrome: The ACTIVIH study. EBioMedicine 2016;8:265–276.
37. Pedro MN, Rocha GZ, Guadagnini D, *et al.*: Insulin resistance in HIV-patients: causes and consequences. Front Endocrinol (Lausanne) 2018;9:514.
38. Pandolfi J, Ferraro A, Lerner M, *et al.*: Purinergic signaling modulates human visceral adipose inflammatory responses: Implications in metabolically unhealthy obesity. J Leukoc Biol 2015;97:941–949.
39. Pandolfi JB, Ferraro AA, Sananez I, *et al.*: ATP-induced inflammation drives tissue-resident Th17 cells in metabolically unhealthy obesity. J Immunol 2016;196:3287–3296.
40. Deng T, Liu J, Deng Y, *et al.*: Adipocyte adaptive immunity mediates diet-induced adipose inflammation and insulin resistance by decreasing adipose Treg cells. Nat Commun 2017;8:15725.
41. Jung S-B, Choi MJ, Ryu D, *et al.*: Reduced oxidative capacity in macrophages results in systemic insulin resistance. Nat Commun 2018;9:1–15.
42. Boutens L, Hooiveld GJ, Dhingra S, Cramer RA, Netea MG, Stienstra R: Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia 2018;61:942–953.
43. Younes S-A, Talla A, Ribeiro SP, *et al.*: Cycling CD4$^+$ T cells in HIV-infected immune nonresponders have mitochondrial dysfunction. J Clin Invest 2018;128:5083–5094.
44. Palmer CS, Ostrowski M, Gouillou M, *et al.*: Increased glucose metabolic activity is associated with CD4+ T-cell activation and depletion during chronic HIV infection. AIDS 2014;28:297–309.
45. Mavigner M, Cazabat M, Dubois M, *et al.*: Altered CD4$^+$ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest 2012;122:62–69.
46. Zevin AS, McKinnon L, Burgener A, Klatt NR: Microbial translocation and microbiome dsybiosis in HIV-associated immune activation. Curr Opin HIV AIDS 2016;11:182–190.
47. Liu J, Williams B, Frank D, Dillon SM, Wilson CC, Landay AL: Inside out: HIV, the gut microbiome, and the mucosal immune system. J Immunol 2017;198:605–614.
48. Pilakka-Kanthikeel S, Kris A, Selvaraj A, Swaminathan S, Pahwa S: Immune activation is associated with increased gut microbial translocation in treatment-naive, HIV-infected children in a resource-limited setting. J Acquir Immune Defic Syndr 2014;66:16–24.
49. Guillén Y, Noguera-Julian M, Rivera J, *et al.*: Low nadir CD4+ T-cell counts predict gut dysbiosis in HIV-1 infection. Mucosal Immunol 2019;12:232–246.
50. Denou E, Lolmède K, Garidou L, *et al.*: Defective NOD2 peptidoglycan sensing promotes diet-induced inflammation, dysbiosis, and insulin resistance. EMBO Mol Med 2015;7:259–274.
51. Grasset E, Puel A, Charpentier J, *et al.*: A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. Cell Metab 2017;25:1075–1090.e5.
52. Kayser BD, Lhomme M, Prifti E, *et al.*: Phosphatidylglycerols are induced by gut dysbiosis and inflammation, and favorably modulate adipose tissue remodeling in obesity. FASEB J 2019;33:4741–4754.
53. Dirajlal-Fargo S, Musiime V, Cook A, *et al.*: Insulin resistance and markers of inflammation in HIV-infected Ugandan children in the CHAPAS-3 trial. Pediatr Infect Dis J 2017;36:761–767.
54. Fjeldborg K, Møller H, Richelsen B, Pedersen S: Regulation of CD163 mRNA and soluble CD163 protein in human adipose tissue in vitro. J Mol Endocrinol 2014;53:227–235.
55. Carreras-Badosa G, Prats-Puig A, Diaz-Roldan F, *et al.*: The macrophage activation product sCD163 is associated with a less favourable metabolic profile in prepubertal children: Soluble CD163 and metabolic profile. Pediatr Obes 2016;11:543–550.
56. Rosso C, Kazankov K, Younes R, *et al.*: Crosstalk between adipose tissue insulin resistance and liver macrophages in nonalcoholic fatty liver disease. J Hepatol 2019;71:1012–1021.
57. Dillon S, Lee E, Kotter C, *et al.*: An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol 2014;7:983–994.
58. Dillon S, Lee E, Kotter C, *et al.*: Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T cell activation in untreated HIV-1 infection. Mucosal Immunol 2016;9:24–37.
59. Geskus RB, Prins M, Hubert J-B, *et al.*: The HIV RNA setpoint theory revisited. Retrovirology 2007;4:65.
60. Farahani M, Novitsky V, Wang R, *et al.*: Prognostic value of HIV-1 RNA on CD4 trajectories and disease progression among antiretroviral-naive HIV-infected adults in Botswana: A joint modeling analysis. AIDS Res Hum Retroviruses 2016;32:573–578.
61. Jacobson DL, Tang AM, Spiegelman D, *et al.*: Incidence of metabolic syndrome in a cohort of HIV-infected adults and prevalence relative to the US population (National Health and Nutrition Examination Survey). J Acquir Immune Defic Syndr 2006;43:458–466.
62. Padmapriyadarsini C, Shet A, Srinivasan R, *et al.*: High prevalence of lipid abnormalities and insulin resistance among antiretroviral naïve HIV-infected children in India. Pediatr Infect Dis J 2018;37:253–257.

63. Davies ML, Johnson MD, Brown JN, Bryan WE, Townsend ML: Predictors of glycaemic control among HIV-positive veterans with diabetes. Int J STD AIDS 2015;26: 262–267.
64. Innes S, Abdullah KL, Haubrich R, Cotton MF, Browne SH: High prevalence of dyslipidemia and insulin resistance in HIV-infected pre-pubertal African children on antiretroviral therapy. Pediatr Infect Dis J 2016;35:e1–e7.
65. Bourke CD, Gough EK, Pimundu G, *et al.*: Cotrimoxazole reduces systemic inflammation in HIV infection by altering the gut microbiome and immune activation. Sci Transl Med 2019;11:eaav0537.
66. McComsey GA, O'Riordan M, Choi J, *et al.*: Mitochondrial function, inflammation, fat and bone in HIV lipoatrophy: Randomized study of uridine supplementation or switch to tenofovir. Antivir Ther 2012;17:347–353.
67. McComsey GA, Daar ES, O'Riordan M, *et al.*: Changes in fat mitochondrial DNA and function in subjects randomized to abacavir-lamivudine or tenofovir DF–emtricitabine with atazanavir-ritonavir or efavirenz: AIDS Clinical Trials Group Study A5224s, Substudy of A5202. J Infect Dis 2013;207:604–611.
68. Samuels R, Bayerri CR, Sayer JA, Price DA, Payne BAI: Tenofovir disoproxil fumarate-associated renal tubular dysfunction: Noninvasive assessment of mitochondrial injury. AIDS 2017;31:1297–1301.

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