Subcutaneous Adipocyte Adenosine Triphosphate Levels in HIV Infected Patients

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Abstract

Lipoatrophy, or fat wasting, remains a syndrome plaguing HIV⁺ patients receiving antiretroviral (ARV) therapy. Both HIV infection *per se* and certain ARV are associated with lowered adipose tissue mitochondrial deoxyribonucleic acid (mtDNA) and mitochondrial ribonucleic acid (mtRNA) levels, but effects on adenosine triphosphate (ATP) production are unclear. We hypothesized that such alterations would accompany lowering of ATP levels in fat of HIV⁺ patients and would be worse in those displaying lipoatrophy. Gluteal-fold, subcutaneous adipose tissue was obtained from HIV seronegative control patients, from HIV⁺ ARV-naïve patients, and those on ARV with or without lipoatrophy. Cellular ATP was measured in isolated adipocytes and preadipocyte fraction cells by bioluminescence. mtDNA copies/cell and oxidative phosphorylation (OXPHOS) mtRNA transcripts were evaluated by quantitative polymerase chain reactions. ATP levels were consistently higher in preadipocyte fraction cells than adipocytes, but values strongly correlated with each other ($r=0.66$, $p<.001$). ATP levels in adipocytes were higher in both ARV-naïve and nonlipoatrophic HIV⁺ patients compared to seronegative controls, but significantly lower in adipocytes and preadipocytes of lipoatrophic versus other HIV⁺ patients. Fat mtDNA copies/cell and OXPHOS mtRNA transcripts were lower in lipoatrophic patient samples compared to HIV seronegative. The ratio of specific OXPHOS transcripts to each other was significantly higher in nonlipoatrophic patients versus all groups, and this ratio correlated significantly with ATP levels in adipocytes. Thus, HIV infection is associated with an increase in adipose tissue ATP stores. Decreases in adipose mtDNA and OXPHOS mtRNA are found in those with HIV on ARV; however, ATP level is effected only in patients displaying lipoatrophy.

Keywords: antiretroviral, fat, lipodystrophy, mitochondria, OXPHOS

Introduction

ADIPOSE TISSUE RESEARCH in HIV⁺ patients has been re-invigorated by recent observations that these tissues act as cure-resistant viral reservoirs that drive chronic immune activation and ensuing HIV-associated “inflammaging,” which is linked to comorbidity progression.^{1–3} Historically, adipose tissue was of interest due to observations of fat redistribution (lipodystrophy), including fat wasting (lipoatrophy), within HIV⁺ patients undergoing antiretroviral (ARV) therapy.⁴ Such lipodystrophy is linked to mitochondrial perturbation induced by both HIV infection itself⁵ and certain nucleoside reverse transcriptase inhibitors (NRTIs),⁶ although the extent each contributes is debatable.^{7,8} Older NRTIs that have been associated with lipoatrophy, such as the thymidine analogs like stavudine (d4T) and zidovudine

(ZDV), are still in use in resource-limited countries resulting in increases in adverse drug reactions⁹ and hypertension,¹⁰ as well as altered drug resistance.¹¹ While multiple mechanisms have been proposed as causes for HIV- and ARV-induced mitochondrial toxicity, the end result of mitochondrial dysfunction is associated with comorbidities such as obesity,¹² nonalcoholic fatty liver disease,¹³ and neurologic disease.¹⁴ Therefore, new data could provide insights into how metabolically-unhealthy adipose tissue impacts inflammaging, such as how extracellular adenosine triphosphate (ATP) in fat tissue promotes apoptosis-resistant, inflammatory T cells^{15,16} or how adipocytes potentially hinder HIV cure efforts.¹⁷

The usage of ARV has been shown to result in mitochondrial perturbations, such as oxidative phosphorylation (OXPHOS) mitochondrial ribonucleic acid (mtRNA)

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transcriptional and translational changes, as well as overall mitochondrial deoxyribonucleic acid (mtDNA) abundance, in both immune cells and adipocytes,^{18,19} with the extent dependent upon ARV regimen.²⁰ Previous studies from our laboratory have shown that both mtDNA per cell¹⁸ and mtRNA ND1 (NADH Dehydrogenase Subunit I) and ND6 (NADH Dehydrogenase Subunit VI) transcript levels⁸ are decreased in subcutaneous (SC) adipose tissue in HIV patients with lipodystrophy; however, no studies have queried ATP production in such tissues. *In vitro* studies do show effects of certain ARV on hepatic,²¹ insulinoma,²² and adipocyte-like cell line²³ in lowering ATP production, possibly resulting in apoptosis,²⁴ and confirming these results *in vivo* is critical to understanding the extent to which specific ARV induces mitochondrial dysfunction.

HIV infection itself alters mitochondrial homeostasis in adipose tissue⁷ and immune cells,^{25,26} but the precise mechanisms and overall effects are also unclear. The HIV accessory protein *vpr* binds to the adenine nucleotide translocator in neuronal mitochondria, leading to decreased mitochondrial membrane potential and decreased ATP synthesis.²⁷ Similar results are found after either HIV1 *tat* or *nef* expression in neuronal cells,²⁸ T cells *in vitro*,²⁹ and an array of tissue-specific mitochondria.³⁰ However, Ogawa *et al.*³¹ indicate the HIV early phase p2 peptide as an activator of mitochondrial cytochrome *c* oxidase, resulting in increased ATP production in human MT4 cells, and Villeneuve *et al.*³² show higher ATP production capacity in HIV-transgenic rats due to altered fatty acid processing and OXPHOS protein translation. Furthermore, they highlight HIV-mediated increases in electron flux through changes to glycolysis rates, as do Palmer *et al.* in human patient T cells,³³ Barrero *et al.* in cultured monocytes,³⁴ and Liao *et al.* in cultured T cells,³⁵ and lipid metabolism may also be important to late stages of HIV infectivity.^{32,36} Thus, the kinetics of metabolic impact(s) HIV infection has upon adipocytes is unclear, especially relating to lipodystrophy etiology.

While a plethora of studies indicate the effects of both HIV infection and certain ARV drugs upon adipocyte mitochondrial homeostasis, the production of ATP, arguably the main function of mitochondria, has not been assessed in relation to lipodystrophy. To better understand functional changes in mitochondrial bioenergetics in adipose tissue, a cross-sectional study was designed to evaluate adipocyte and preadipocyte fraction ATP levels, mitochondrially-encoded OXPHOS RNA and DNA levels, and potential relationships to HIV, use of older NRTIs (ZDV, d4T, or didanosine [ddI]), and lipodystrophy status.

Materials and Methods

A single site cross-sectional study conducted at the Research Clinic of the Hawaii Center for AIDS, John A. Burns School of Medicine, University of Hawaii (UH), USA was approved by the UH Committee on Human Subjects, and informed consent documents were signed by all participants.

Study population and clinical assessments

Consenting patients were recruited into the following four cohorts: (1) HIV-seronegative controls: recruited primarily from friends and partners of HIV-infected patients seen at the

Research Clinic of the Hawaii Center for AIDS; (2) ARV-naïve cohort: HIV-infected individuals with no history of ARV; (3) nonlipodystrophic cohort: HIV-infected individuals on similar NRTI containing ZDV, ddI, tenofovir (TDF), or abacavir (ABC) therapy for more than 6 months, with no self-reported lipodystrophy; and (4) lipodystrophic cohort: HIV-infected individuals on older NRTI-containing (ZDV, d4T and/or ddI) therapy for more than 6 months, with self-reported and investigator-confirmed lipodystrophy. Subjects with an acute illness within 2 weeks of beginning the study, persistent or unstable chronic infections, AIDS-defining illnesses or illnesses other than HIV, history of diabetes mellitus or hypogonadism, or use of anabolic agents, glucocorticoids, appetite stimulant, or lipid-lowering agents within 2 months before study entry were excluded.

Eligible patients who met all screening criteria were seen for a single study visit. Medical history, including type and duration of all ARV used, were obtained. Body composition was assessed by whole body Dual Energy X-ray Absorptiometry (DEXA) utilizing a GE Lunar Prodigy scanner. Blood was obtained in a fasting state, defined as nothing by mouth except water for ≥ 12 h before the blood draw, and was assayed in a local Clinical Laboratory Improvement Amendments and College of American Pathologists-certified laboratory for complete blood count, HIV-1 RNA quantification by polymerase chain reaction (PCR; Ultrasensitive Roche Amplicor Monitor Version 1.0 assay), CD4 cell count, chemistries, and metabolic assays, including fasting glucose, insulin, total cholesterol, and triglyceride levels. Blood samples for glucose and venous lactate measurement were collected in sodium fluoride tubes, maintained on ice and processed within 30 min. Insulin resistance (IR) was calculated by homeostasis model assessment of insulin resistance (HOMA-IR).

SC adipose tissue was collected from the gluteal fold of the lateral thigh by an open biopsy technique. Following betadine prep and local 1% lidocaine injection and using sterile techniques, a 1.5 cm linear incision was made along the gluteal fold of the lateral buttock-thigh area. Approximately 300 mg of fat was removed and immediately placed in Dulbecco's phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) on ice. The wound was closed with 2-3 resorbable sutures.

Adipocyte and preadipocyte isolation

Approximately 180 mg of adipose tissue was minced into smaller pieces and digested in collagenase type CLS (Sigma-Aldrich, St. Louis, MO) in PBS containing BSA (Sigma-Aldrich) at 37°C for ~ 90 min, or until the tissue was $\sim 90\%$ digested, shaking periodically. Digested tissue was filtered through a 300 μ m nylon mesh to remove large undigested pieces of tissue and centrifuged at 400 g for 10 min at 4°C to fractionate cells. The top layer contained adipocytes, the middle layer contained cell debris and excess collagenase, and the pelleted cells consisted of preadipocytes, immune and other resident cells, and erythrocytes. Adipocytes were collected with a transfer pipette and washed twice with PBS on ice. The cell pellet was resuspended in erythrocyte lysis buffer (G-Biosciences, Maryland Heights, MO) consisting of NH_4Cl , KHCO_3 , and EDTA and incubated for 10 min. The remaining cells, herein deemed preadipocyte fraction, were

re-pelleted by centrifugation and washed twice with $1 \times$ PBS on ice. Adipocytes were verified by staining and counting cells with Oil red O, and $98\% \pm 3\%$ of the cells had lipid droplets. Oil red O positivity was lacking in $95\% \pm 5\%$ of the cells in preadipocyte fraction. Adipocyte and preadipocyte fraction viability was determined using Trypan blue exclusion.

Total cellular ATP quantification

ATP levels in isolated preadipocytes and adipocytes were measured using an ATP Bioluminescence HSII Kit (Roche Applied Sciences, Indianapolis, IN). Serial dilutions from 10^{-6} to 10^{-11} M of the ATP nucleotide were prepared to generate a standard curve. Approximately 10^4 to 10^7 adipocytes or preadipocytes were isolated, resuspended in dilution buffer with 1:1 volume of lysis buffer, and allowed to incubate at room temperature for 5 min. Samples were assayed in triplicate in dark 96-well plates. Luciferase reagent was added and ATP luminescence measured using a Veritas luminometer (Turner BioSystems, Sunnyvale, CA). Samples were also tested using the ApoGlow BioAssay (Cambrex Bio Science, Rockland, ME) to control for ATP hydrolysis effect, and final ATP concentrations are reported after normalization using sample ADP/ATP ratio and cell count.

mtDNA copies/cell

Total DNA was isolated from ~ 30 mg of SC adipose tissue immediately following biopsy using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., Gaithersburg, MD). Standardization of quantitative polymerase chain reactions (qPCRs) was performed using LightCycler FastStart DNA Master SYBR Green I (Roche) with a Roche LightCycler instrument (Roche), as described previously.³⁷ Briefly, each sample and standard were run in duplicate ($20 \mu\text{L}$ reaction volume) and contained: SYBR Green Master Mix, mitochondrial NADH Dehydrogenase Subunit II (ND2), or genomic Fas Ligand (FasL) primers (Idaho Technology, Inc., Salt Lake City, UT) and sample DNA. PCR cycling conditions were: denaturation at 95°C for 10 min, 35–40 amplification cycles at 95°C for 10 s, 58°C for 5 s, and 72°C for 5 s. Commencement of PCR was immediately followed by melt curve analysis beginning at 65°C and increasing temperature half a degree every 30 s for 60 cycles. Results were analyzed with Version 4.0 LightCycler software (Roche), and mtDNA copies/cell were calculated by multiplying the mean C_p value of ND2 reactions by 2 and dividing by the mean C_p value of FasL reactions.

mtRNA levels of OXPHOS transcripts

Total RNA was isolated from ~ 90 mg piece of adipose tissue that was immediately stored in RNeasy Later at -70°C using RNeasy Lipid Tissue Kit (Qiagen). RNA quality was checked and quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Purified RNA was reverse transcribed to cDNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche) on a PCR Thermal Cycler instrument (Eppendorf, Westbury, NY). OXPHOS gene expression was measured by qPCR on a LightCycler instrument with a LightCycler TaqMan Master Kit (Roche). Standard curves ranging from 10^1 to 10^3 copies were prepared from a single recombinant plasmid containing the ND1, ND6, Cy-

tochrome B (CytB), and the nuclear housekeeping ribosomal L13 gene. Sequences of gene-specific primers and probes were customized according to Galluzzi *et al.*³⁸ Each reaction was conducted in duplicate ($20 \mu\text{L}$ reaction volume) containing: sample DNA, TaqMan Master Mix (FastStart Taq DNA polymerase, reaction buffer, dNTP mix, MgCl_2), L13, CytB, ND1, or ND6 primer (Idaho Technology), corresponding L13, CytB, ND1, or ND6 probe (Idaho Technology), and ddH_2O . PCR cycling conditions and OXPHOS gene quantification were conducted as previously described.⁸ Mean ND1, ND6, and CytB copies were reported as a ratio to the mean L13 concentration for each sample.

Statistical analyses

Differences between cohorts were analyzed utilizing chi-square for dichotomous values or Mann–Whitney *U* non-parametric test for two independent samples. Correlations between parameters were assessed using Spearman's rank correlation coefficient after log transformation of data. A two-sided *p* value $< .05$ was used to determine statistical significance. All statistical analyses were performed using the SPSS version 15 (SPSS, Inc., Chicago, IL).

Results

Clinical assessments and metabolic characteristics

A total of 39 patients participated in this study: 9 HIV-seronegative, 6 HIV⁺ ARV-naïve, 8 HIV⁺ nonlipoatrophic, and 16 HIV⁺ lipoatrophic patients. The demographic, immunovirologic, body composition, and metabolic characteristics of the cohorts are summarized in Table 1. The cohorts were similar in age, gender, and body mass index. Lipoatrophic patients had significantly lower percentages of extremity fat by DEXA compared to HIV-seronegative patients and tended to be lower than nonlipoatrophic patients, although significance was not reached. No differences were found between lipoatrophic and nonlipoatrophic patients in CD4 count, HIV RNA, current ARV use, or in the duration of exposure to NRTIs, non-NRTIs, or protease inhibitors.

Mitochondrial parameters

Median values for adipocyte ATP, preadipocyte fraction cell ATP, mtDNA copies/cell, and OXPHOS transcript levels from each cohort are reported in Table 2. Adipocyte and preadipocyte ATP levels and mtDNA levels by cohort are also depicted graphically in Figure 1. Median ATP levels in preadipocytes (1.24 – 11.75 pM/cell) were significantly higher compared to adipocytes (0.04 – 1.13 pM/cell). Adipocyte ATP was significantly higher in ARV-naïve ($p = .05$) and nonlipoatrophic ($p = .01$) patients compared to seronegative controls. Adipocyte ATP was significantly lower in lipoatrophic patients compared to ARV-naïve ($p = .005$) and to nonlipoatrophic patients ($p = .001$). Adipocyte and preadipocyte fraction ATP levels were similar in HIV-seronegative controls compared to lipoatrophic patients ($p = .275$; $p = .83$, respectively). In preadipocytes, cellular ATP levels were significantly reduced in lipoatrophic patients compared to ARV-naïve ($p = .02$) and nonlipoatrophic ($p = .04$) individuals.

Median mtDNA copies/cell were significantly lower in lipoatrophic compared to ARV-naïve ($p = .04$) and seronegative controls ($p \leq .001$). Median mtDNA copies/cell were

TABLE 1. MEDIAN OF PATIENT DEMOGRAPHICS, ANTIRETROVIRAL REGIMENS AND DURATION, BODY COMPOSITION, AND METABOLIC CHARACTERISTICS IN HIV SERONEGATIVE, HIV⁺ ANTIRETROVIRAL-NAIVE, HIV⁺ NONLIPOATROPHIC, AND HIV⁺ LIPOATROPHIC COHORTS

	<i>HIV seronegative</i>	<i>HIV⁺ antiretroviral naive</i>	<i>HIV⁺ nonlipoatrophic</i>	<i>HIV⁺ lipoatrophic</i>
Number of participants, <i>n</i>	9	6	8	16
Age, years	43 (17.5)	44.5 (12.5)	43.5 (19.0)	51 (14.3)
Male, <i>n</i> /Female, <i>n</i>	8/1	7/0	7/0	15/1
Body mass index, kg/m ²	26.7 (5.1)	26.6 (4.0)	24.0 (5.2)	25.3 (4.5)
Extremity fat by DEXA as percent of total fat (%)	40% (8%) [#]	34% (19%)	31% (18%)	27% (13%) [#]
No. of patients on NRTI at time of study (d4T/ZDV/ddI/TDF/ABC)	—	—	0/6/1/2/1	6/8/1/5/1
Exposure time to NRTI, years	—	—	6.0 (4.3)	5.5 (3.5)
No. of patients on NNRTI at time of study/Exposure time to NNRTI, years	—	—	7/3.0 (5.0)	11/5 (5.0)
No. of patients on PI at time of study/ Exposure time to PI, years	—	—	2/8.5 (0.0)	6/5.5 (8.0)
CD4, cells/mm ³	—	476 (489)	380 (254)	522 (340)
HIV RNA (No. of patients <50 copies/mL)	—	0	8	14
ALT, IU/L	26 (14)	39 (36)	39 (36)	29 (31)
Fasting total cholesterol, mg/dL	212 (56) ^{*#}	170 (53) [*]	195 (36)	167 (70) [#]
Fasting triglycerides, mg/dL	72 (50) ^{*#}	158 (75) [*]	104 (131)	176 (90) [#]
Fasting lactate, mg/dL	0.8 (0.6) [*]	1.0 (0.9)	1.0 (1.5)	1.5 (0.7) [*]
Fasting glucose, mg/dL	94 (14)	91 (30)	92 (13)	86 (26)
HOMA-IR	1.5 (0.8)	1.5 (2.2)	0.9 (0.2)	1.7 (1.7)

^{*#}Statistically significant with $p < .05$ by Mann–Whitney U test.

() Standard deviation.

ABC, abacavir; ALT, alanine transaminase; d4T, stavudine; ddI, didanosine; DEXA, Dual Energy X-ray Absorptiometry; HOMA-IR, homeostasis model assessment of insulin resistance; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-NRTI; PI, protease inhibitor; TDF, tenofovir; ZDV, zidovudine.

also decreased in nonlipoatrophic compared to seronegative controls ($p = .002$). Evaluation of mtRNA transcripts by qPCR demonstrated that lipoatrophic patients had significantly lower relative amounts of transcripts of ND1/L13 ($p < .05$), ND6/L13 ($p < .05$), and CytB/L13 ($p < .05$) expression levels compared to seronegative controls. In nonlipoatrophic patients, ND1/L13 ($p < .05$) and CytB/L13 ($p < .05$) transcripts were lower compared to HIV seronegatives. However, the ratio of the OXPHOS transcripts ND1 to CytB was significantly higher in nonlipoatrophic subjects

compared to lipoatrophic ($p < .001$), ARV-naive ($p < .05$), and HIV-seronegative ($p < .001$) patients.

Analyses determined that adipocyte ATP levels correlated significantly with those in preadipocyte fraction cells ($r = 0.66$, $p < .001$). No correlations were found between adipocyte or preadipocyte ATP levels and mtDNA, ND1/L13, ND6/L13, or CytB/L13 levels. However, a significant correlation was found between adipocyte ATP and ND1/CytB ($r = 0.50$, $p = .002$), and a correlation trended toward significance in preadipocyte ATP and ND1/CytB ($r = 0.32$, $p = .06$).

TABLE 2. MEDIAN OF ADIPOCYTE AND PREADIPOCYTE ADENOSINE TRIPHOSPHATE, MITOCHONDRIAL DEOXYRIBONUCLEIC ACID, AND MITOCHONDRIAL RIBONUCLEIC ACID TRANSCRIPTION LEVELS IN HIV-SERONEGATIVE, ANTIRETROVIRAL-NAIVE, NONLIPOATROPHIC, AND LIPOATROPHIC COHORTS

	<i>HIV seronegative (n = 9)</i>	<i>ARV naive (n = 6)</i>	<i>Nonlipoatrophic (n = 8)</i>	<i>Lipoatrophic (n = 16)</i>
Adipocyte ATP, pM/cell	0.05 (0.28) ^{*#}	1.13 (3.02) ^{#◇}	0.56 (8.06) ^{*^}	0.04 (0.13) ^{◇^}
Preadipocyte ATP, pM/cell	4.44 (6.87)	11.75 (8.30) [#]	10.38 (17.36) [*]	1.24 (7.10) ^{*#}
mtDNA, copies/cell	1,784 (889) ^{#^}	1,366 (1,704) [*]	586 (793) [#]	523 (412) ^{*^}
ND1/L13	14.3 (9.7) ^{*#}	8.8 (10.6)	7.7 (10.2) [*]	4.7 (6.6) [#]
ND6/L13	6.2 (7.4) [#]	2.6 (5.1)	4.4 (6.9)	1.4 (2.4) [#]
CYTB/L13	12.0 (11.3) ^{*#}	7.0 (8.0)	3.8 (5.9) [*]	4.1 (3.9) [#]
ND1/CYTB	1.1 (0.3) ⁺	1.3 (0.8) [*]	2.2 (0.7) ^{+*^}	1.3 (0.5) [^]

^{*#◇} Statistically significant at $p < .05$ by Mann–Whitney U test.

^{^+} Statistically significant at $p < .001$ by Mann–Whitney U test.

() Standard deviation.

ARV; ATP, adenosine triphosphate; CYTB, Cytochrome B; mtDNA, mitochondrial deoxyribonucleic acid; ND1, NADH Dehydrogenase Subunit I; ND6, NADH Dehydrogenase Subunit VI.

FIG. 1. Box-and-Whisker plots of log transformed (a) adipocyte ATP, (b) preadipocyte ATP, and (c) mitochondrial DNA levels in HIV-seronegative, ARV-naive, nonlipoatrophic, and lipoatrophic cohorts. Twenty-fifth percentile quartiles (*lower box section*), medians (*horizontal line drawn through*), 75th% quartiles (*upper box section*), and standard deviations indicating maximum and minimum values are depicted. ARV, antiretroviral; ATP, adenosine triphosphate.

No significant correlations were found between adipocyte or preadipocyte ATP and metabolic or body composition parameters; however, a weak correlation was seen between adipocyte ATP and HOMA-IR ($r=0.34$, $p=.05$). Analyses involving HIV⁺ lipoatrophic and nonlipoatrophic samples only showed a trend toward significance between adipocyte ATP and % extremity fat ($r=0.39$, $p=.08$) and between preadipocyte ATP and % extremity fat ($r=0.42$, $p=.07$). In addition, a correlation between preadipocyte ATP and trunk fat ($r=0.48$, $p=.04$) or with total fat ($r=0.51$, $p=.026$) was seen (trunk fat and total fat, data not included).

Discussion

In this study, the data indicate that adipocyte and preadipocyte ATP levels are elevated during HIV infection but lowered in patients on ARV with lipoatrophy. Surprisingly, both HIV⁺ cohorts on ARV had reduced fat mtDNA levels and mitochondrially-encoded ND1 and CytB RNA transcript levels, which are integral for OXPHOS, but ATP levels were only lower in those patients displaying significantly lower

percentages of extremity fat, as measured by DEXA, compared to seronegative controls.

Overall, the data agree with past studies regarding changes to mtDNA and OXPHOS RNA levels in lipoatrophic and ARV-treated patients.^{39–42} ATP levels were higher in ARV-naive and ARV-treated nonlipoatrophic HIV⁺ patients and did not coincide with an increase in mtDNA or mtRNA levels. It has been shown that HIV-infected cells display altered cellular metabolic profiles,^{32,43} that activated T cells produce more ATP,⁴⁴ and that immune cells in HIV-infected persons display elevated PD1 expression,⁴⁵ which is associated with exhausted phenotypes⁴⁶ and greater spare respiratory capacity.⁴⁷ Thus, it is possible that both intrinsic and extrinsic mechanisms affecting post-transcriptional and post-translational modifications of OXPHOS enzymes are influencing ATP production during HIV infection. Interestingly, the ratio of transcripts for OXPHOS subunits ND1 to CytB was highest in nonlipoatrophic patients, which correlated to ATP levels, potentially indicating compensatory mechanisms against HIV-induced electron leak⁴⁸ or cytochrome *c* inhibition³⁰ to maintain mitochondrial homeostasis.

ATP levels were uniformly higher in preadipocyte fraction cells compared to adipocytes, which are consistent with the greater energy demands of preadipocytes.⁴⁹ It is also possible that the presence of immune cells^{50,51} (observed by histology and cell sorting, data not shown) or other cell types such as endothelial and vascular cells within the preadipocyte fraction may have contributed to their higher ATP levels. Detectable HIV DNA levels within monocytes in the periphery and pro-inflammatory cytokine expression within macrophages in adipose tissue have been shown to be higher in lipoatrophic and HIV⁺ patients, respectively,⁵¹ potentially influencing ATP levels directly or through cytokine signaling.^{52,53} Interestingly, Shikuma *et al.*¹⁸ showed that OXPHOS protein levels correlated between circulating immune cells and adipose tissue, suggesting that peripheral blood mononuclear cell (PBMC) levels could be indicative of lipoatrophy status. However, no studies have extended this correlation to ATP levels as yet, although ATP levels have been assessed in PBMC of HIV⁺ patients.^{54,55} In this study, cohort-related changes in ATP levels occurred in tandem in both adipocyte and preadipocyte fractions, with increases in ATP levels seen together with unsuppressed HIV infection and lack of peripheral fat tissue loss. Results suggest that factors likely to affect ATP levels, such as HIV, ARV therapy, and/or alterations associated with the presence of lipoatrophy, affect both cell populations equally. Thus, further examination of adipocyte and preadipocyte fraction ATP levels and correlation to lipodystrophy progression are warranted.

Total ATP levels were lower in lipoatrophic patients compared to ARV-naïve or nonlipoatrophic HIV⁺ patients, but were similar to seronegative individuals. *In vitro* studies by Viengchareun *et al.* showed that 3T3 brown adipocytes exposed to ZDV in culture led to significant reduction of ATP²³; however, the potentially confounding effect of concurrent HIV infection was not assessed. The lowered ATP levels seen in these lipoatrophic patients cannot be attributed exclusively to the use of older NRTIs as both ARV-treated cohorts did not differ significantly in the use or duration of ARV medications, but only in the degree of peripheral SC fat loss. However, we cannot rule out that ATP levels in lipoatrophic and HIV-seronegative individuals may be resulting from genetic predisposition, host immunologic factors, or various hormonal and metabolic factors not assessed in this study. Thus, the assessment of individual patients longitudinally would help to address whether accumulation of adipocyte mtDNA damage, changes in mtRNA transcriptional regulation, or other intrinsic and extrinsic mechanisms of induced mitochondrial dysfunction may predispose to lipodystrophy.

Limitations of this study include the cohort size and cross-sectional approach. While the study was limited by relatively small cohort sizes, which reduced statistical power, and by small biopsy tissue amounts, which precluded measuring mtDNA copies/cell and mtRNA strictly within adipocytes and/or preadipocyte fractions at the cellular level compared to at the whole adipose tissue level, significant conclusions could be drawn. The cross-sectional nature of this study precludes any clear identification as to whether such decreases in ATP associated with lipoatrophy are the cause or effect of decreased SC adipose content. One could hypothesize that excessive reactive oxygen species production⁵⁶ as a result of HIV- or ARV-induced mitochondrial dysfunction, in

conjunction with a pro-inflammatory cytokine milieu driven by excess ATP,¹⁶ could result in enhanced adipocyte apoptosis, potentially coinciding with lipoatrophy progression.^{57,58}

In conclusion, this study reports for the first time that HIV infection is associated with increase in adipose tissue ATP stores without a preceding rise in mtRNA OXPHOS transcripts or in mtDNA levels, whereas ATP store depletion in lipoatrophic fat tissue is accompanied by decreases in mtDNA and mtRNA. Together, these observations warrant larger and more intensive longitudinal studies to elucidate how such mitochondrial changes influence lipoatrophy and inflammaging in patients with HIV.

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Author Disclosure Statement

Dr. Gerschenson has been a consultant for Abbott and Oncolys Biopharma and currently consults for Cardax, Inc. Dr. Shikuma has received training and research support from Pfizer, Gilead, and Merck Pharmaceuticals and has consulted for Glaxo Smith Kline. The other authors have no conflicts of interests.

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