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Mitochondrial RNA and DNA alterations in subcutaneous fat tissue of HIV+ subjects with lipoatrophy are linked to antiretroviral therapy and not to HIV infection

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Abstract

Objective—Assess the effect of ART versus HIV on fat mitochondria.

Methods—Subcutaneous-fat was collected from 45 HIV-infected subjects on ART with lipoatrophy, 11 HIV-infected ART-naïve, and 9 healthy-controls. Three mt-transcripts NADH-dehydrogenase subunit 1(ND1), cytochrome-b (CYTB), and NADH-dehydrogenase subunit 6 (ND6) genes were quantitated using Taqman-probes, and normalized to nuclear-encoded ribosomal L13.

Results—ND1/L13 and CYTB/L13 were reduced in HIV-ART-lipoatrophy versus ART-naïve [3.4 vs. 7.2; p=0.017 and 2.5 vs. 4.6; p=0.006]. No difference was found between naïve and controls (p >0.70). ND6/L13 was similar between all groups. DEXA-measured limb fat (grams) and fat-mtDNA (copies/cell) were also lower in HIV-ART-lipoatrophy versus HIV-infected ART-naïve [4382 vs. 7662; p=0.02 and 726 vs. 1372; p=0.03], but no difference was found between ART-naïve and controls. In a multiple regression analysis, limb fat correlated with all 3 mtRNA, while mtDNA did not correlate with mtRNAs or limb fat.

Conclusion—In contrast to ART-naive, patients with HIV-ART-lipoatrophy had significant depletion in fat-mtDNA and mtRNAs. This suggests that mitochondrial toxicity in lipoatrophy may be driven by ART and not by HIV itself. In addition, mtRNA abnormalities, and not mtDNA depletion, may be a key driving force behind lipoatrophy.

Keywords

adipose; mitochondria; mitochondrial DNA; mitochondrial RNA; lipoatrophy; lipodystrophy

INTRODUCTION

Mitochondrial abnormalities have been described in HIV-infected subjects with peripheral fat loss or lipoatrophy (1–5). Thus far, the pathogenesis of mitochondrial dysfunction in HIV patients remains unclear, and the degree to which antiretroviral therapy (ART), specifically nucleoside analogues reverse transcriptase inhibitors (NRTI) are responsible, as opposed to HIV infection itself is not known. In addition, although mitochondrial DNA (mtDNA)

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depletion has been frequently described in the fat of HIV infected subjects with lipoatrophy (1–5), limited data exist on mitochondrial RNA (mtRNA) and its relationship to changes in mtDNA and peripheral fat.

Several factors including NRTI use, lower nadir CD4, older age, and higher body mass index have been shown in HIV infected individuals to correlate with mitochondrial disease, whether in the form of hyperlactatemia or lipoatrophy (6,7). To our knowledge, in the HIV-infected population no studies have concomitantly investigated mtRNA and DNA as well as objective assessment of lipoatrophy. Also, no such study has included both ART naïve group and uninfected groups as controls, and none investigated the relationship of mtRNA and mtDNA to HIV disease and treatment factors. Thus, in this study, we measured mtRNA and mtDNA levels in adipose tissue of HIV infected lipoatrophy group, ART naïve group and HIV uninfected group in order to explore the relationship of these mitochondrial indices to HIV and treatment factors.

Methods

Study design/population

This is a single site, cross sectional, controlled observational study. Patients were enrolled between May 2005 and April 2007. We studied a group of HIV-infected adults referred to the Case HIV Metabolic Center for assessment of lipoatrophy. For the HIV lipoatrophy/ART group, the inclusion criteria were HIV infection, age ≥ 18 years, receipt of stable antiretroviral therapy containing a thymidine analogue NRTI for at least 6 consecutive months prior to study entry, HIV-1 RNA < 50 copies/mL and clinical lipoatrophy at study entry. Clinical lipoatrophy was defined by self report by the study subjects of fat loss in at least 2 of the following areas: face, arms, legs, and buttocks, and confirmed by the investigator. We also included an HIV+ ART-naïve group which enrolled subjects who had documented HIV infection and no prior ART. As controls, we enrolled an HIV negative healthy control group who were undergoing cosmetic surgical procedures and who were similar in age to the HIV+ groups. Exclusion criteria included current opportunistic infections, renal or hepatic impairment, coagulation problems, abnormal PT/PTT or platelets < 75, diagnosis of diabetes, active endocrine disorders, and history of recent use of hormonal therapies. All patients were enrolled at the John T Carey Special Immunology Unit of Case Medical Center, Cleveland, Ohio and signed a written informed consent approved by the Institutional Review Board of University Hospitals of Cleveland, Cleveland, Ohio.

Study Evaluations—Study evaluations in HIV-infected subjects included clinical examination, blood sampling, and a whole body Dual Energy X-ray Absorptiometry (DEXA) scanning for body fat composition (Hologic 4500). Blood was drawn in fasting state (after at least 8 hours fast) for lipid panel, insulin, glucose, and lactate levels. Lactate levels were all drawn in fasting state, without a tourniquet or fist clenching, and were immediately processed. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated from fasting plasma glucose and insulin values (8). An experienced surgeon performed excisional biopsies of subcutaneous fat from the lower abdomen of all study subjects under local anesthesia (using lidocaine without epinephrine). All biopsies were collected with the written informed consent of subjects, and with the approval of the Institutional Review Board of University Hospitals of Cleveland, Cleveland, Ohio.

Mitochondrial DNA Measurements—Analysis of mtDNA copies/cell was conducted by absolute quantitative real-time PCR as previously described (4,9). DNA was extracted from subcutaneous adipose tissue biopsies (stored at −70°C in RNAlater) using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc., USA). Total DNA was quantified in ng/μl using a UV-

Vis ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington DE) and integrity examined by agarose gel electrophoresis. Standardization of real-time PCR was performed using LightCycler FastStart DNA Master Plus SYBR Green I (Roche Applied Sciences, Indianapolis, IN) with a LightCycler instrument (Roche). A dilution series ranging from 10^7 to 10^3 copies/cell of the control plasmid containing the 90 bp mtDNA NADH dehydrogenase subunit II (mitochondrial) and 98 bp Fas Ligand (genomic) gene was prepared for each standard (10). The mitochondrial primers (Idaho Technologies BioChem, ID), mtDIR (CAC AGA AGC TGC CAT CAA GTA) and mtREV (CCG GAG AGT ATA TTG TTG AAG AG) were specific for a region of the mitochondrial protein NADH Dehydrogenase subunit II. The nuclear genomic primers (Idaho Technologies BioChem, ID), GenDIR (GGC TCT GTG AGG GAT ATA AAG ACA) and GenREV (CAA ACC ACC CGA GCA ACT AAT CT) were specific for the nuclear region of the genome encoding for Fas Ligand. Each sample and standard were run in duplicate (20 μl reaction volume) containing: SYBR Green Master Plus Mix (FastStart Taq DNA polymerase, reaction buffer, dNTP mix, SYBR Green 1 dye, $MgCl_2$), 10 pM mitochondrial or genomic forward and reverse primers, and approximately 10 ng DNA from sample. PCR cycling conditions were: 95°C for 10 minutes followed by 35–40 cycles of 95° C for 3 seconds (denaturation), 58°C for 5 seconds (annealing), and 72°C for 5 seconds (extension). At the conclusion of the PCR, a melt curve analysis was immediately begun with the following conditions: beginning at 65°C, the temperature increased half a degree every 30 seconds for 60 cycles. Samples were run in duplicate and results were then analyzed with Roche Version 4.0 LightCycler software. Absolute mtDNA copies/cell for each sample was calculated by dividing mean mtDNA values by mean genomic DNA values, and then multiplying by 2.

Mitochondrial RNA measurements

Total RNA was isolated from subcutaneous adipose tissue biopsies stored at −70C in RNALater (Sigma-Aldrich, St. Louis MO.) using RNeasy Lipid Tissue Kit (Qiagen Inc.). 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 Applied Sciences) on a Polymerase Chain Reaction (PCR) Thermal Cycler instrument (Eppendorf, Westbury NY). Oxidative phosphorylation (OXPHOS) gene expression was then measured using real-time PCR on a LightCycler with a Lightcycler TaqMan Master Kit (Roche Applied Sciences).

Standard curves ranging from 10^7 to 10^3 copies were prepared from a single recombined plasmid containing the NADH Dehydrogenase I (ND1), NADH Dehydrogenase VI (ND6), Cytochrome B (CytB), and the nuclear housekeeping ribosomal L13 gene (11). Each different transcript was quantified in a separate reaction to avoid non-specific amplifications and to ensure maximum reliability. Each reaction was conducted in duplicate (20 μl reaction volume) containing: approximately 1μL cDNA from sample, TaqMan Master Mix (FastStart Taq DNA polymerase, reaction buffer, dNTP mix, $MgCl_2$), 0.5 μM L13 or CytB or ND1 or ND6 primer (BioChem), 0.2 μM corresponding (L13, CytB, ND1, or ND6) probe (Sigma-Aldrich), and ddH_2O. Sequences of genespecific primers and probes were customized according to Galluzzi et al. (11). PCR cycling conditions were: 95°C for 15 minutes followed by 40–45 cycles of 95° C for 10 seconds (denaturation), 57°C for 15 seconds (annealing), and 72°C for 15 seconds (extension). Following the final PCR cycle, a step of 37°C for 30 seconds allowed the instrument to cool to room temperature. Fluorescence of each transcript was detected at a wavelength of 530 nm. Quantification of ND1, ND6, and CytB copies were calculated by the ratio of each mitochondrial transcript mean concentration to the mean L13 concentration for each sample.

Statistical methods

Demographics, clinical characteristics and mitochondrial parameters are described by study group for all groups, and HIV-related characteristics are described for HIV-infected patients only. Continuous measures are described by medians and ranges, and nominal variables are described with percents. Continuous measures were compared using Wilcoxon rank sum tests, and nominal variables using Chi squared analysis or Fisher's exact test as appropriate.

The relationships between the outcome measures of mtRNAs and variables of interest were examined. Spearman's rank correlation coefficients were calculated for each of the variables with each of the mtRNAs measures. Since the correlations with limb fat mirrored the correlations with limb fat/BMI, we elected to present only the correlations with the latter variable. Regression models were developed separately for each of the mtRNAs measures and for mtDNA separately in a two-stage approach. The goal was to include disease, treatment and biological variables in the same model (correcting for age, BMI and limb fat), while being cognizant of the sample size limitations. In the first stage four models were constructed: one including race and gender, one including disease-related variables, one including treatment-related variables and the last including laboratory values. Variables were chosen either on the basis of the bi-variate results, or because they were known to have clinical significance. Each model also included age at study entry, BMI and limb fat. Any variable from each of the first stage models which was significant at the $p \leq 0.2$ level, or lacking that, the most significant variable in the model, was included in the second stage analyses All analyses were carried out using SAS v. 9/1 (The SAS Institute, Carey, NC). The level of significance for was set at 0.05.

RESULTS

Study Population

Between May 2005 and April 2007, 65 subjects enrolled in this study; 45 subjects with established HIV lipoatrophy on ART, 11 HIV-infected ART-naïve and 9 uninfected controls. Table 1 summarizes the demographics and baseline characteristics of all study participants. There were 45 (69%) males enrolled, 54% were Caucasians, 36% African-Americans, and 8% Hispanics. Overall, the median age and BMI were 45 years and 26 kg/m^2 in the HIV+ group versus 43 years and 40 kg/m^2 in the HIV-group ($p= 0.87$ and 0.002, respectively for between-group differences in age and BMI). Subjects with HIV-lipoatrophy/ART were receiving antiretroviral therapy containing at least one NRTI for median (range) duration of 79 (6–186) months at the time of study evaluation. Median duration of PI therapy was 17 (0–106) months. At the time of study evaluations, 10 (22%) of subjects were on d4T containing therapy, 35 (78%) on AZT, 18 (40%) a PI-based regimen, and 22 (49%) an NNRTI based regimens. At study entry, median CD4+ cell count was 576 cells/mm^3 (range 67–1427) in the lipoatrophy/ART group and 275 cells/mm^3 (194–434) in the ART naïve group ($p<0.001$). All lipoatrophy/ART subjects had HIV-1 RNA< 50 copies/mL, while the ART-naïve group had median HIV-1 RNA of 31,850 (range 364–>100,000) copies/mL. Median nadir CD4+ cell count (cells/mm^3) was lower in the lipoatrophy/ART group when compared to ART-naïve [194 (138–544) vs. 275 (194–325); $p<0.0001$].

Between group comparisons of mitochondrial and metabolic indices

Table 2 summarizes the metabolic and mitochondrial results in all study groups. The lipoatrophy/ART group had higher fasting triglycerides and cholesterol when compared to ART naïve [168 (46–1522) mg/dL vs. 96 (49–420) mg/dL; $p=0.003$ for triglycerides, and 182 (110–305) mg/dL vs. 150(101–165) mg/dL for cholesterol; $p=0.0001$], but similar lactate, insulin, and HDL-cholesterol. HOMA-IR was higher in the lipoatrophy/ART vs. ART naïve group [2.3 (0.1–8.1) vs. 0.8 (0.1–4.7); $p=0.048$]. DEXA-measured limb fat (grams) and limb fat/BMI were significantly lower in the HIV+ lipoatrophy/ART group when compared to the

ART-naïve [4382 (2158–12734) grams vs. 7662 (2668–18313) grams; p=0.02 and 170 (92–354) vs. 320 (100–620); p=0.005]. When separately looking at fat composition of upper and lower extremities, only leg fat was significantly lower in the lipoatrophy/ART group (2952 (1293–9210) grams vs. 6226(1612–14825) grams in ART-naive; p=0.005). Similarly, levels of fat mtDNA (copies/cell) were lower in HIV+ lipoatrophy/ART versus HIV+ naïve [726 (194–3091) vs. 1372 (532–2721); p=0.03]. No difference was found in fat mtDNA between HIV+ naïve and healthy controls (p=0.79).

The expression of ND1, ND6, and CYT B were all different although they strongly correlated among each other (for example correlation between ND1 and ND6 r=0.90; p<0.0001; between ND1 and CYTB r=0.96; p<0.0001). In each of the groups, ND1 was always expressed at higher values than ND6 and CYTB, and the ratio of ND1/ND6 was preserved and similar in all groups (p>0.54). However, ND1/CYT B was lower in the ART-naïve group when compared to the lipoatrophy group [1.25 (0.69–1.61) vs. 1.59 (0.0002–2.82); p=0.03], but similar to the healthy controls (p=0.59).

ND1/L13 and CYTB/L13 were significantly reduced in HIV+ lipoatrophic subjects on ART when compared to HIV+ naïve [3.4 (0.01–16.6) versus 7.2 (3–15.4); p=0.017 and 2.5 (0.03–17) versus 4.6 (1.9–22.3); p=0.006]. However, no differences were found between HIV+ naïve and healthy controls (p=0.70 and 0.94, respectively for ND1/L13 and CYTB/L13). ND6/L13 was similar between all groups (p>0.14).

Correlation Analysis

In the lipoatrophy/ART group, significant correlation was found between limb fat/BMI and ND1/L13 (r=0.36; p=0.026), CYTB/L13 (r=0.35; p=0.028), and ND6/L13 (r=0.41; p=0.009). No correlation was found between fat mtDNA and any of the 3 mtRNAs or between fat mtDNA levels and limb fat/BMI. A significant correlation was found between HDL (but not total) cholesterol and ND1/L13 (r=0.35; p=0.02), CYTB/L13 (r=0.42; p=0.004), and ND6/L13 (r=0.38; p=0.01). Triglyceride levels correlated with CTYB/L13 (r=−0.36; p=0.01). Levels of mtRNAs or mtDNA did not correlate with either nadir or current CD4, HIV duration or any duration or type of therapy, lactate, insulin or HOMA-IR.

In the ART-naïve group, no correlations were found between mtDNA and mtRNA levels, or between either of the mitochondrial indices and limb fat/BMI. Only BMI negatively correlated with ND1/L13 (r=−0.78; p=0.0075), ND6/L13 (r=−0.85; p=0.0016) and CYTB/L13 (r=−0.72; p=0.019), but not with fat mtDNA.

Regression analysis

In the final, second-stage analyses, only limb fat remained significant in the models for ND1, ND6 and CYT B (p=0.04, 0.03 and 0.009, respectively), while race remained significant for only ND1/L13 (p=0.06). Age, gender, BMI, nadir or current CD4, duration or type of therapy did not. In a similar model for predictors of fat mtDNA levels; only the duration of thymidine NRTIs and duration of PI therapy (p=0.002 and 0.02, respectively) predicted fat mtDNA levels, whereas limb fat, age, gender, race, BMI, and HIV disease factors did not.

DISCUSSION

To our knowledge, this is the first study that assessed mitochondrial RNA expression along with mtDNA levels in the fat tissue of HIV infected subjects with lipoatrophy and which included an ART naïve group as well as an HIV negative control group. Few prior studies have assessed the effects of ART on mtRNAs (11–14), and only one (11) was performed on HIV-infected subjects. In this report, we show that both mtRNA and mtDNA are significantly

decreased in HIV infected adults with lipoatrophy when compared to either HIV-infected ART naïve group or an uninfected control group. Interestingly no differences were found between HIV+ ART-naïve group and HIV-negative controls, suggesting that these mitochondrial disturbances are the result of ART and not to HIV infection itself. Earlier reports have suggested that HIV infection itself might cause mtDNA decline in ART-naïve subjects, but this was shown mostly in peripheral blood mononuclear cells, and not in adipose tissue (15,16). In this study, the different groups were closely matched by age. This matching is crucial for the success of any such trial aimed at studying the effect of HIV and/or treatment on mitochondrial indices in HIV infected adults since increasing age may be associated with more mitochondrial abnormalities (17) which may significantly confound the study results in the absence of careful matching. Also, our HIV negative group had significantly higher BMI, which makes our results even stronger since obesity has been linked to mitochondrial abnormalities (18), and therefore the higher BMI in our population may have attenuated the observed differences in mitochondrial abnormalities between the studied groups.

Mitochondrial DNA depletion in HIV infected treated subjects with lipoatrophy has been consistently described (1–5,19). However, mitochondrial dysfunction is not always associated with mtDNA depletion (20,21), and severe mtDNA depletion has been reported in asymptomatic subjects (22,23). Thus, additional mechanisms for mitochondrial alterations in HIV beyond only mtDNA depletion are likely, as recently suggested (20,24). To our knowledge only one prior small study assessed mtRNA levels in adipose tissue of 11 HIV+ patients with lipoatrophy and 7 HIV uninfected controls (11). The mtRNA levels in that study were also reduced in HIV+ when compared to HIV negative controls, but no HIV+ group without lipoatrophy was included, making it impossible to differentiate between the effects of HIV infection versus those of ART. The importance of mtRNA alterations, independently of mtDNA depletion, has been suggested by prior cell culture work. Galluzzi et al have previously shown that NRTIs can induce a significant decrease in mtRNA levels in cell lines, even before any noticeable mtDNA depletion (12). Similarly, d'Amati et al have shown a significant disruption of mitochondrial cristae and alteration of mtRNA, but no change in mtDNA levels after 4 weeks of AZT treatment of mouse muscle cells (13). In our study, the lack of correlation between mtRNA alteration and mtDNA levels suggest that the alteration in transcription is not secondary to changes in mtDNA but is rather a primary effect of therapy. Interestingly, only the mitochondrial heavy chain transcripts, ND1 and CYTB, were decreased in HIV-infected treated subjects with lipoatrophy and not the light chain transcript ND6. This suggests that mitochondrial heavy strand transcription is affected but not light chain transcription. In addition, the fact that limb fat/BMI correlated with mtRNA but not mtDNA also suggests that mtRNA alteration may indeed be the primary mitochondrial alteration that only at times coexists with mtDNA depletion. The study reported by Mallon et al support this hypothesis (14). In that study, Mallon reported a significant decrease in mtRNA production after 2 weeks of NRTI in healthy HIV uninfected adults (14). This occurred before any changes in mtDNA levels of fat mass.

On regression analysis, only limb fat and limb fat adjusted to BMI (limb fat/BMI) correlated with all three mtRNA measurements while HIV disease factors like CD4 cell count and duration of HIV infection did not. This again supports our observation that the mtRNA alterations in HIV infected with lipoatrophy are linked to the use of antiretroviral therapy. The duration of thymidine NRTIs correlated with fat mtDNA levels although PI duration was also independently correlated. Limb fat/BMI did not correlate with mtDNA levels.

As expected, HIV-infected treated subjects with lipoatrophy had higher fasting triglycerides, cholesterol, HOMA-IR when compared to ART naïve. Additionally, correlation analysis showed that HDL cholesterol correlated with all three mitochondrial transcripts. Mitochondrial function is sensitive to fat (25), insulin, and glucose levels (26). The mitochondria maybe

uncoupled due to the increased fat and this is corresponding with decreased transcription. Insulin resistance is associated with decreased ATP production. Thus, there maybe multiple etiologies for the mitochondrial dysfunction

One limitation of our study is the lack of HIV-infected treated control group without lipoatrophy. We have recently learned that it is difficult to fully exclude lipoatrophy by clinical assessment alone in HIV patients. Indeed, studies have shown that despite the lack of clinical diagnosis of lipoatrophy, HIV infected women and men have lower amount of peripheral fat when compared to gender- and age-matched HIV negative subjects (27,28), suggesting that clinical lipoatrophy may only be the extreme form of peripheral fat loss in HIV patients and that the subclinical form may be more common than previously thought. Thus a group of HIV treated without lipoatrophy is challenging to enroll but would need to be explored in future studies. In addition, incorporating detailed mitochondrial indices in future ART-switch studies of subjects with lipoatrophy would be helpful in dissecting the effect of ART in general from that of lipoatrophy.

In summary, a significant decrease in mtDNA levels and in mtRNAs were found in HIV infected subjects on ART with lipoatrophy, but not in ART-naïve subjects. This strongly support that ART, and not HIV infection, play the major role in the generation of mitochondrial disturbances in HIV lipoatrophy. The correlation found between limb fat/BMI and mtRNA and not mtDNA, support the fact that mtRNA alteration, and not mtDNA depletion, may be the primary mitochondrial disturbance in HIV lipoatrophy.

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Table 1

Demographics, clinical and HIV-related characteristics in HIV infected patients and controls

Parameter Median (range)	Lipoatrophy (n=45)	ARV naïve (n=11)	Healthy controls (n=9)
Age (years)	48(31–74)**	32(25–45)	43(30–64)$^{\beta}$
Sex (male)	84%	55%	11%$^{\beta}$
Race			
White	58%*	27%	67%$^{\beta}$
African Americans	33%	55%	22%
Hispanic	7%	9%	11%
Other	2%	9%	0
Known duration of HIV (months)	127(26–252)**	4(1–66)	NA
Nadir CD4+(cells/mm3)	199(30–544)**	275(194–325)	NA
Duration of NRTI (months)	79(6–186)	0	NA
Duration of thymidine NRTI (months)	61(6–186)	0	NA
Duration of NNRTI therapy (months)	17(0–113)	0	NA
Duration of PI therapy (months)	17(0–106)	0	NA
CD4+ cell count at time of evaluation (cells/mm3)	576(67–1427)**	194(275–434)	NA
Median HIV-1 RNA (copies/mL)	< 50	31,850(364–>100,000)	NA
Antiretrovirals used at study entry		NA	NA
Stavudine	22%		
Zidovudine	78%		
Abacavir	33%		
Tenofovir	20%		
Lamivudine/emtricitabine	91%		
PI	40%		
NNRTI	49%		
Entry Inhibitor	2%		

NA=not applicable for that group; PI= protease inhibitor; NNRTI= non-nucleoside analogue reverse transcriptase inhibitor

For comparison between HIV+ lipoatrophy and HIV+ naïve groups

* p<0.05

# p<0.01

** p<0.001

For comparison between HIV+ naïve and uninfected controls

β p<0.05

Table 2

Mitochondrial and Metabolic Parameters in HIV-Infected Subjects and Uninfected Controls

Parameter Median (Range)	HIV Lipoatrophy (n=46)	HIV+ ART-naïve (n=11)	Uninfected controls (n=9)
BMI	26.1(19.0–41.7)	26.1(18.2–32.2)	39.9(21.3–53.4)$^{\beta}$
Total Cholesterol (mg/dL)	182(110–305)**	150(101–165)	NM
HDL-cholesterol (mg/dL)	34(14–68)	28(19–57)	NM
Triglycerides (mg/dL)	168(46–1522)$^{\#}$	96(49–420)	NM
Insulin (mIU/L)	11(1–39)	4(1–18)	NM
HOMA-IR	2.3(0.1–8.1)*	0.8(0.1–4.7)	NM
DEXA-trunk fat (grams)	7482(2878–28580)	6236(1854–17607)	NM
DEXA-limb fat (grams)	4382(2158–12734)*	7662(2268–18313)	NM
Limb fat/BMI	170(92–354)	320(100–620)	NM
Total Lean Body Mass (grams)	60584(24035–81067)*	48415(30087–71474)	NM
Fat mtDNA (copies/cell)	726(194–3091)*	1372(532–2721)	1344(588–2677)
ND1/L13	3.4(0.01–16.6)*	7.2(3.0–15.4)	9.1(5.0–18)
ND6/L13	0.93(0.01–8.9)	1.9(0.4–13.3)	1.9(0.4–18)
CYTB/L13	2.5(0.03–17)$^{\#}$	4.6(1.9–22.3)	7.3(0.5–13)

ND=not measured

For comparison between HIV+ lipoatrophy and HIV+ naïve groups

* p<0.05

$^{\#}$ p<0.01

** p<0.001

For comparison between HIV+ naive and uninfected controls

$^{\beta}$ p<0.05