Human Immunodeficiency Virus Treatment–Induced Adipose Tissue Pathology and Lipoatrophy: Prevalence and Metabolic Consequences

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Background. Lipoatrophy and metabolic complications of treatment of human immunodeficiency virus (HIV) infection may share common associations with adipose tissue pathology and inflammation. To investigate these relationships, we undertook a large-scale study of adipose tissue, body composition, and metabolic outcomes among HIV-infected adult men at a tertiary hospital HIV cohort during the period 2001–2007.

Methods. Assessments included adipose biopsies ($n = 211$) for investigation of adipocyte mitochondrial DNA content, adipocytokine expression, and adipose macrophage content; and whole-body dual-energy x-ray absorptiometry (DEXA) scans ($n = 225$) for objective body composition changes; 138 individuals contributed both biopsy and DEXA data.

Results. Compared with 78 treatment-naive control subjects, 98 zidovudine recipients (48%) and 49 stavudine recipients (67%) had leg fat measures <10% threshold value. Adipose samples associated with current stavudine or zidovudine ($n = 99$) revealed significant adipocyte mitochondrial DNA depletion, adipose tissue macrophage infiltration, and elevated proinflammatory cytokine levels, compared with samples from control subjects and nonthymidine nucleoside reverse-transcriptase inhibitor (NRTI) recipients (all $P<.05$). Improvements in adipose pathology after NRTI switching ($n = 21$ longitudinal samples) correlated with increased preswitch adipose inflammation and less severe fat loss (both $P<.05$). Elevated ratios of total to high-density lipoprotein cholesterol levels and Homeostatic Metabolic Assessment scores correlated independently with lipoatrophy severity ($P<.05$) and increased body mass index ($P<.05$) in thymidine NRTI-experienced individuals. No effect of demographic or HIV-related variables, or HIV protease inhibitor therapy exposure was detected.

Conclusions. Adipose tissue pathology and lipoatrophic fat loss are highly prevalent among recipients of stavudine- or zidovudine-based HIV treatment and are associated with adverse metabolic outcomes. Restoring adipose tissue health appears to be an important issue in the long-term treatment of this patient population.

A syndrome of progressive lipoatrophy affecting human immunodeficiency virus (HIV)–infected patients was first identified >10 years ago [1, 2], coinciding with the introduction of highly active antiretroviral treatment (HAART). Although initially ascribed to HIV protease inhibitor treatment [1], clinical studies from the early HAART era indicated that use of stavudine and zidovudine nucleoside reverse-transcriptase inhibitors (NRTIs) was a dominant risk factor [2–4]. Subsequent clinical trials [5, 6] and cohort studies [7, 8] have established strong associations between stavudine treatment and risk of lipoatrophy, whereas zidovudine treatment is associated with the syndrome to a lesser extent [2–4, 9]. These findings are consistent with studies of adipose biopsy samples that identified specific effects of stavudine and zidovudine on mitochondrial DNA content, mitochondrial function, and adipose pathology [10–13].

There has been a significant reduction in lipoatrophy incidence in recent years associated with decreasing use of stavudine and zidovudine, although the persistent nature of established lipoatrophy, with only partial recovery after switching or removing NRTI drugs [14, 15], has ensured that the syndrome remains highly prevalent. For example, 49% of HIV-infected men in the Fat Redistribution and Metabolic Change in HIV

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Infection (FRAM) study had measures of leg subcutaneous fat mass below the lowest 10% of control values [7], whereas 26% of cohort participants in the Data collection on Adverse events of anti-HIV Drugs (D:A:D) study had subjective evidence of lipodystrophy [16]. Given that loss of ~30% subcutaneous fat volume is required before lipoatrophy becomes clinically apparent [17], these data suggest a large burden of adipose tissue pathology among treatment-experienced HIV-infected patients [11].

Lipoatrophy can be stigmatizing and has been associated with depression and reduced quality of life [18, 19]. However, it is also apparent that lipoatrophy is associated with endocrine dysfunction (reviewed in [20]), in keeping with the pivotal role of adipose tissue in regulating whole-body metabolism [21]. Thus, lipoatrophy has been independently associated with elevated cardiovascular disease risk [22], incident diabetes mellitus [23], and pro-atherogenic dyslipidemia [24]; this suggests that lipoatrophy and obesity may share common pathological features and adverse metabolic consequences, despite their obvious phenotypic differences.

In this study, we examined relationships between HIV therapy, adipose tissue pathology, mitochondrial DNA depletion, objectively assessed fat loss, and metabolic outcomes among participants in the Western Australian HIV Cohort study

SUBJECTS AND METHODS

Study design. Adult male participants in the Western Australian HIV Cohort study were enrolled in this nonrandomized, observational study during the period 2001–2007. Multiple biopsy samples were contributed from a total of 58 participants. Patients were sampled longitudinally either (1) at the time of being antiretroviral therapy [ART] naive and subsequently after receiving thymidine NRTI ($n = 15$), (2) at the time of being ART naive and subsequently after receiving nonthymidine NRTI ($n = 15$), or (3) before and after switching NRTI therapy ($n = 28$). Overall, 211 biopsy specimens were collected for analysis (48 samples from ART-naïve patients, 105 from thymidine NRTI recipients, 17 from patients who initially received nonthymidine NRTI therapy, and 41 from recipients of nonthymidine NRTIs with a prior history of thymidine NRTI therapy).

We used whole-body dual-energy x-ray absorptiometry (DEXA) scan measurements of body composition among 78 treatment-naive participants, as well as 98 patients treated exclusively with zidovudine-based HAART and 49 treated with stavudine-based HAART. Relationships between fat loss and adipose tissue mitochondrial DNA depletion were further investigated in a subset of patients with both biopsy and DEXA data (39 ART-naive patients, 64 zidovudine-treated patients, and 33 stavudine-treated patients).

Study participants continued to receive routine clinical care with regular clinic reviews every 3 months, and study participation did not influence HIV treatment decisions. Clinical and laboratory data routinely collected included age, race, weight, body mass index (BMI), T cell subset levels, and history of antiviral drug use. All study procedures were approved by the Royal Perth Hospital Bioethics Committee, and subjects provided written consent.

Body composition assessments. Whole-body DEXA scans were performed on a QDR-4500A scanner (Hologic). To evaluate lipoatrophy, we focused on an objective measure of peripheral subcutaneous fat (leg fat as a percentage of leg mass) normalized to body mass index, as described elsewhere [12, 29]. We also evaluated leg fat mass (in kilograms) and an alternative measurement used in the analysis of the FRAM cohort [7], in which the percentage of leg fat was normalized only to height (in meters) squared.

Subcutaneous adipose tissue biopsies and adipocyte mitochondrial DNA analysis. Biopsy specimens were obtained from the suprailiac region, and mitochondrial and nuclear DNA copy numbers were measured by real-time polymerase chain reaction, using Taqman chemistry on the 7900 sequence detection system platform (Applied Biosystems), as previously described [11, 25]. This assay has been validated in an international quality assurance study [26].

Tissue adipokine analysis. Frozen tissue (50–100 mg) was manually homogenized with 200μl of phosphate buffered saline containing protease inhibitors. Homogenates were centrifuged at 25,160*g* (Allegra 25R) for 10 minutes at 4 degrees. Samples were stored at −80°C prior to quantitation of levels of adiponectin (dilution of 0.02 of tissue homogenate) and IL-8 and leptin (neat homogenate) by LINCOplex kit immunoassay (human adipocyte kit, HADCYT-61K) on a luminex 200 platform (Luminex, Australia). Quantification of each protein was performed using standard curves generated in each run. As measures of homogenate quantity could not be standardized across individuals adipokine results were expressed as ratios of one to another.

Adipose tissue immunostaining. Tissue histology was assessed in paraffin-processed biopsy sections of 4-μm thickness. The number of macrophages (expressing CD68 or MAC387 antigens) and the expression of cytokines (interleukin [IL]–6, IL-8, IL-12, IL-18, tumor necrosis factor, and interferon-γ) were evaluated using standard immunohistochemical techniques. Primary antibody was applied for 1 h. Details of the source and individual concentrations of antibodies used are available from the authors. A Chemicon kit (catalogue number Det-HP-1000) that employed an initial blocking step with goat serum and carrier protein and a 2-step streptavidin-horseradish peroxidase combination were used for immunohistochemistry, prior to colormetric detection by 3,3′-diaminobenzidine tetrahydrochloride.

Quantitative assessment of cytokine expression in the cy-

Table 1. Last Available Body Composition Data for Adult Human Immunodeficiency Virus–Infected Men Who Were Antiretroviral (ART) Naive or Who Were Treated Exclusively with Zidovudine (AZT) or Stavudine (d4T)

Characteristic	ART-naive participants	Zidovudine recipients	Stavudine recipients
No. of DEXA scans	78	98	49
Duration current backbone NRTI therapy, months	…	45 (25–73)	22 (15–40)
Lamivudine as second NRTI agent, percentage of patients	…	94	78
Percentage of protease inhibitor recipients	…	34	69
Age at DEXA scan, years	42 (32–50)	43 (39–53)	40 (37–46)
Age at time of commencement of ART, years	…	38 (33–46)	37 (35–44)
CD4 T cell count, cells/μL			
At time of DEXA scan	299 (146–508)	556 (301–796)	550 (342–703)
At commencement of ART	…	318 (160–478)	320 (148–435)
Percentage of patients with undetectable viral load	3	75	59
Leg fat level, kg	4.8 (3.6–6.7)	3.6 (2.8–4.9)[a]	2.7 (2.1–3.8)[a]
Leg fat percentage/BMI	0.84 (0.7–1.0)	0.65 (0.5–0.8)[a]	0.55 (0.4–0.8)[a]
BMI	23.9 (20.8–26.5)	24.2 (22.0–26.4)	22.0 (20.2–24.4)
Trunk fat, kg	6.5 (4.2–10.3)	7.5 (4.9–10.3)	5.5 (3.8–8.4)
Triglyceride level, mmol/L	1.4 (0.9–1.9)	1.5 (1.0–2.6)	1.9 (1.2–2.8)[b]
Lactate level, mmol/L	1.3 (1.0–1.8)	1.5 (1.2–2.0)	1.4 (1.2–1.9)
Total cholesterol level, mmol/L	4.1 (3.5–7.2)	4.9 (4.3–5.6)[a]	5.5 (4.6–6.3)[a]
LDL cholesterol level, mmol/L	2.4 (1.7–3.2)	2.7 (2.2–3.3)[c]	3.1 (2.4–3.9)[a]
HDL cholesterol level, mmol/L	1.0 (0.8–1.1)	1.1 (0.9–1.4)[a]	1.1 (0.9–1.4)[b]
Ratio of total to HDL cholesterol level	4.1 (3.5–5.6)	4.3 (3.4–5.4)	4.7 (3.7–5.7)
HOMA score	1.6 (1.1–3.2)	2.1 (1.2–4.2)	1.7 (1.1–3.0)

NOTE. Data are median (interquartile range), unless otherwise indicated. BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); DEXA, dual-energy x-ray absorptiometry; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NRTI, nucleoside reverse-transcriptase inhibitor.

[a] $P<.001$, for comparisons with the ART-naive group, by t test with transformation of data (as appropriate).
[b] $P<.01$, for comparisons with the ART-naive group, by t test with transformation of data (as appropriate).
[c] $P<.05$, for comparisons with the ART-naive group, by t test with transformation of data (as appropriate).

toplasm of adipocytes was evaluated under light microscopy with a 7-point staining score that took both intensity and extent of immunoreactivity into account. The derived score for each case ranged from 0 (negative staining) to 3 (maximum staining intensity detected in 100% of cells). Macrophage count was performed per $\times 40$ field of view, and the mean value for $\geqslant 2$ fields was recorded.

All metabolic measures in plasma samples were obtained by Pathwest Laboratory (Perth, Western Australia) using standard methods. For analyses of insulin resistance, Homeostatic Metabolic Assessment (HOMA) scores were calculated as follows: [fasting serum insulin level (in mU/L) $\times$ fasting plasma glucose level (mmol/L)]/22.5.

Statistical analysis. Simple correlations were summarized using the Spearman correlation coefficient, and sample subgroups were compared by t tests; additional assessments of association were undertaken in a linear regression framework, with log-transformation of variables as appropriate. Mixed-effects models were used for analyses with multiple measures per person. Analyses were performed using S-Plus, version 8.1 (Tibco).

RESULTS

The characteristics of the body composition (DEXA) dataset are described in Table 1, including demographic, clinical, and ART-related variables. The adipose tissue biopsy dataset, including the distribution of HIV treatment regimens at the time of biopsy collection, is shown in Table 2. Biopsy samples were used for analyses of adipose tissue mitochondrial DNA depletion ($n = 211$), adipose tissue morphology ($n = 68$), and measurements of tissue cytokines by histochemistry ($n = 69$) and protein immunoassay ($n = 67$), to assess the effects of starting or altering HIV therapy on these variables.

Body Composition and Adipose Tissue Pathology among ART-Naive Subjects

We first sought to establish normative values for ART-naive study participants, to examine potential effects of HIV disease status and demographic variables on body composition and adipose tissue pathology.

Proportional fat distribution. This analysis considered 78 male ART-naive individuals with a median age of 42 years,

Table 2. Characteristics of the Adipose Tissue Biopsy Dataset for 211 Biopsy Specimens

Characteristic	ART-naive patients	Thymidine NRTI-naive patients[a]	Zidovudine recipients	Stavudine recipients	Thymidine-experienced patients[a]
No. of biopsy samples	49	16[b]	66	33	39[c]
Duration of current backbone NRTI therapy, months	…	5.9 (5.3–6.5)	40.6 (10.5–61.8)	35.8 (10.6–48.0)	7.0 (5.5–11.4)
Lamivudine as second NRTI agent, percentage of patients	…	100	98	79	95
Percentage of protease inhibitor recipients	…	44	39	45	23
Age at time of biopsy, years	41 (34–50)	44 (40–47)	47 (39–53)[d]	44 (38–52)	52 (42–57)[e]
CD4 T cell count, cells/μL					
At time of biopsy	345 (160–493)	614 (504–804)	624 (496–780)[e]	578 (437–809)[e]	609 (480–759)[e]
At commencement of ART	…	418 (238–553)	359 (180–475)	357 (304–480)	345 (154–530)
Percentage of patients with an undetectable viral load	4	94	85	76	90
Adipocyte mitochondrial DNA level, copies/cell	882 (609–1653)	967 (676–1601)	513 (349–954)[e]	263 (180–376)[e]	710 (337–1098)

NOTE. Data are median (interquartile range), unless otherwise indicated. ART, antiretroviral therapy; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); NRTI, nucleoside reverse-transcriptase inhibitor.

[a] Patients received abacavir and/or tenofovir.
[b] Biopsy samples were available for 15 abacavir recipients and 2 tenofovir recipients (1 patient received abacavir and tenofovir concurrently).
[c] Biopsy samples were available for 33 abacavir recipients and 8 tenofovir recipients (2 patients received abacavir and tenofovir concurrently).
[d] $P<.05$, for comparisons with the ART-naive group, by t test with transformation of data (as appropriate).
[e] $P<.001$, for comparisons with the ART-naive group, by t test with transformation of data (as appropriate).

median current $CD4^+$ T cell count of 299 cells/μL, and a median current plasma HIV RNA level of 4.9 $\log_{10}$ copies/mL (Table 1). In this group, BMI-adjusted leg fat percentages were log-normally distributed, with a median value of 0.84. This measure was not associated with BMI ($P = .8$), age ($P = .3$), $CD4^+$ cell count ($P = .5$), or HIV load ($P = .4$). In contrast, both leg fat mass and an alternative measure of fat distribution, percentage of leg fat normalized by height-squared, were found to be significantly associated with BMI ($R = 0.72$ and $R = 0.55$, respectively; $P<.001$), $CD4^+$ T cell count ($R = 0.36$ and $R = 0.23$, respectively; $P<.05$) and HIV RNA level ($R = -0.33$ and $R = -0.24$, respectively; $P<.04$). For this reason, subsequent analyses focused on BMI-normalized percentage of leg fat.

Adipocyte mitochondrial DNA content. Forty-six evaluable biopsy specimens collected before commencement of HIV therapy provided a median adipocyte mitochondrial DNA content of 882 copies/cell (Table 2). There was no detectable effect of age ($P = .7$), $CD4^+$ T cell count ($P = .7$), or HIV RNA level ($P = .4$) on this variable, and there was also no evidence of an association between mitochondrial DNA content and fat distribution in 38 subjects with data on both measures available ($P = .5$).

Adipose tissue pathology and cytokine expression. Adipose tissue macrophage content ($n = 20$) and analyses of adipocytokine expression ($n = 13$) among individuals who were untreated for HIV infection were used as baseline comparator for treatment effects. Blinded evaluation of adipose samples by an experienced pathologist [25] demonstrated no evidence of pathological change.

Plasma lipids and insulin resistance. Results of fasting metabolic assessments for the ART-naive group are provided in Table 1. Measures of BMI-adjusted leg fat correlated marginally with HOMA scores ($R = 0.23$; $P = .08$) but not with the available corresponding measures of plasma lipids: levels of triglycerides ($P = 0.4$), lactates ($P = .8$), total cholesterol ($P = .9$), low-density lipoprotein cholesterol ($P = .3$), or high-density lipoprotein (HDL) cholesterol ($P>.9$).

Effects of Thymidine NRTI-Based Regimens on Body Composition and Adipose Tissue

Proportional fat distribution and cumulative thymidine NRTI therapy. Here we considered 147 male West Australian HIV cohort participants who had been treated exclusively with stavudine ($n = 49$; median duration of exposure, 22 months) or zidovudine ($n = 98$; median duration of exposure, 45 months) (Table 1). In this group, the last available on-treatment leg fat measures were considerably lower than those in ART-naive control subjects ($P<.001$) for both zidovudine-treated and stavudine-treated patients, although there was little difference in trunk fat or overall BMI across these groups (Table 1). Comparing these results to the ART-naive population, 48% of zidovudine-treated and 67% of stavudine-treated patients had values less than the first decile of the naive group (Figure 1*A*).

More severe lipoatrophy correlated with cumulative thymi-

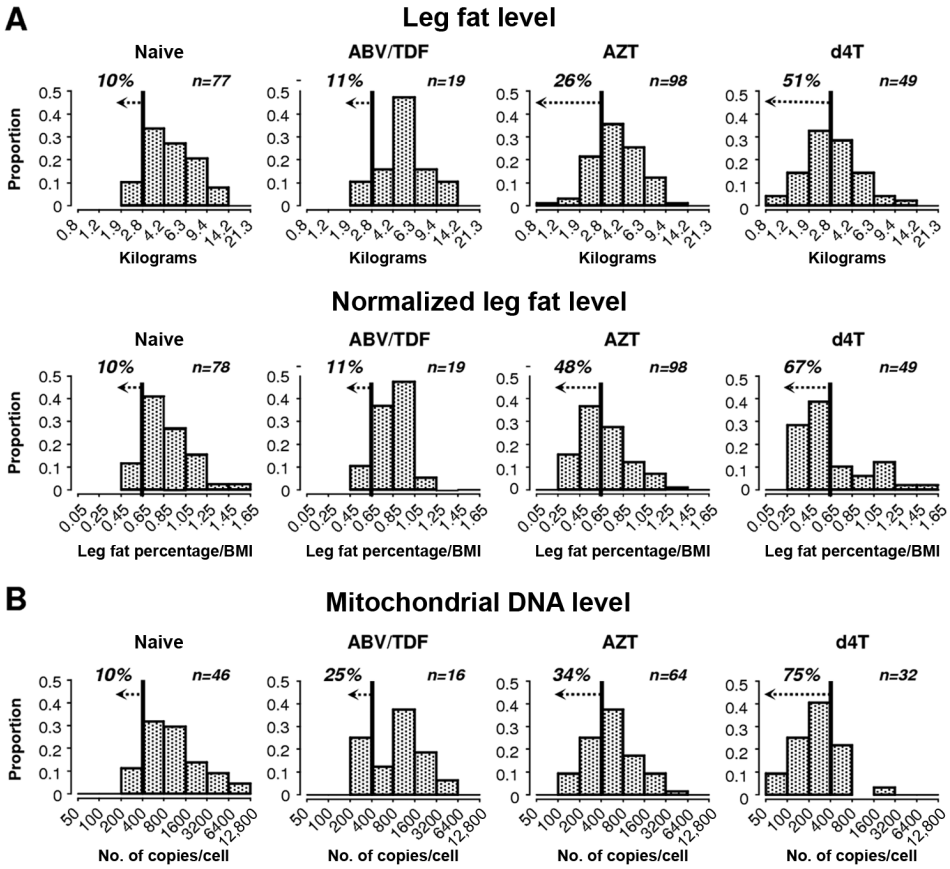

Figure 1. Comparisons of proportional fat distribution (*A*) and adipocyte mitochondrial DNA content (*B*), according to treatment group. In each plot, the cutoff value for the lowest decile in the control group (antiretroviral therapy–naive patients) group is shown, with the proportion of values below this cutoff value indicated for each group. ABV, abacavir; AZT, zidovudine; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); d4T, stavudine; TDF, tenofovir.

dine NRTI exposure (R = 0.27; $P<.001$), higher CD4 T cell count (R = 0.20; $P = .02$), and older age (R = 0.18; $P = .03$), whereas no detectable association was observed with BMI (R = 0.12; $P = .1$) or cumulative protease inhibitor exposure (R = −0.14; $P = .09$). With use of longitudinal models that included all available body composition measurements (496 DEXA scans), we found cumulative zidovudine and stavudine exposure to be the dominant predictor of leg fat percentage ($P<.001$), even with adjustment for CD4 T cell count ($P = .09$), age ($P = 0.3$), HAART component (for cumulative protease inhibitor exposure, $P = .1$), or BMI ($P>.9$). This association between cumulative thymidine NRTI exposure and reduced BMI-adjusted leg fat percentage was also evident in the adipose biopsy dataset (R = 0.55; $P<.001$).

Adipocyte mitochondrial DNA content. Median adipocyte mitochondrial DNA content was reduced by 44% with stavudine use ($n = 32$; $P<.001$) and by 24% with zidovudine treatment ($n = 64$; $P<.001$) (Figure 1*B*). These effects were evident within 2–12 months (stavudine, 10 patients $P<.001$; zidovudine, 12 patients [$P = .04$]), during which period leg fat measures remained stable in the stavudine group ($P = .4$) and increased marginally in the zidovudine group ($P = .05$), as expected [6, 7]. Levels of mitochondrial (mt) DNA remained consistently low thereafter ($P<.009$), with no significant trend associated with longer times on treatment (for 12–60 months, 12 stavudine and 27 zidovudine recipients; for >60 months, 11 stavudine and 27 zidovudine recipients) (Figure 2).

No significant independent effect of HIV protease inhibitor versus NNRTI therapy was observed; in particular, regimens containing efavirenz ($n = 11$) and lopinavir/ritonavir ($n = 18$) were very comparable ($P = .7$). Furthermore, the on-treatment differences were not associated with pretreatment CD4 cell count ($P = .4$), HIV load ($P = .4$), or BMI ($P = .2$).

Effects on adipose tissue. This analysis revealed an evolving pattern of adipose tissue pathology associated with thymidine NRTI treatment characterized by increased tissue macrophage count and proinflammatory cytokine staining (Figure 2). Loss of leg fat and severity of mtDNA depletion correlated independently with both adipose tissue macrophage count (leg fat, R = −0.65 and $P<0001$; mtDNA, R = −0.51 and $P = .004$),

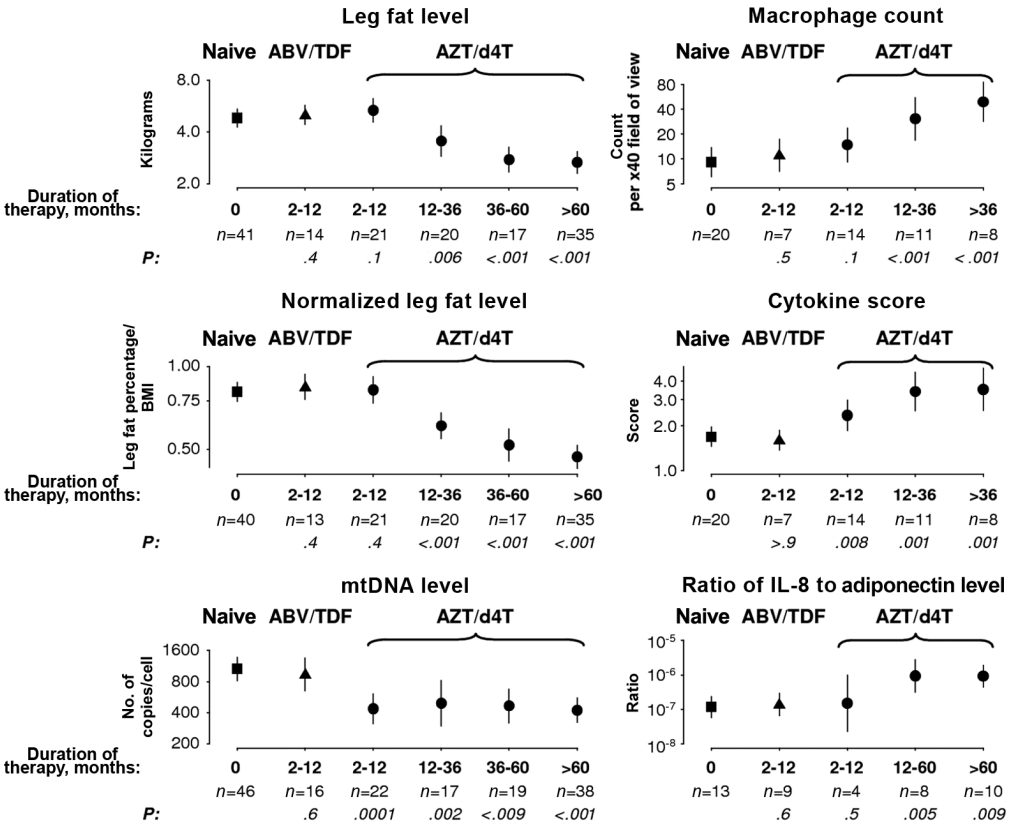

Figure 2. Effects of nucleoside reverse-transcriptase inhibitor treatment on markers of adipose tissue pathology. Mean values ± 2 standard errors (from a linear mixed effects model) are plotted for each marker. *P* values are for comparisons with antiretroviral treatment–naive control subjects. ABV, abacavir; AZT, zidovudine; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); d4T, stavudine; IL-8, interleukin-8; TDF, tenofovir.

and pro-inflammatory cytokine score (normalized percentage of leg fat, R = −0.48 and $P = .005$; mtDNA, R = −0.59 and $P < .001$). Moreover, significant changes were observed within 18 months after initiation of therapy (a 112% increase in macrophage count [$P = .02$] and a 53% increase in mean cytokine score [$P = .009$]) in 11 patients with both pretreatment and on-treatment measures available. Increased adipose tissue ratios of IL-8 to adiponectin level and of IL-8 to leptin level were also associated with cumulative thymidine exposure (Figure 2) and correlated with lipoatrophy severity (normalized percentage of leg fat, R = −0.44 [$P = .04$] and R = −0.54 [$P = .009$], respectively) (Figure 3).

Effects on plasma lipids and insulin resistance. Here, we used available database records of fasting lipid levels for the 104 participants treated exclusively with zidovudine ($n = 69$) or with stavudine ($n = 35$). As shown in Table 1, the overall effect of thymidine NRTI treatment on these fasting metabolic variables was relatively modest, with nonsignificant increases in the ratio of total to HDL cholesterol level and the HOMA score measure of insulin resistance. However, additional analysis revealed significant associations between lipoatrophy severity and increased levels of serum lactate (R = −0.25; $P = .01$) and triglycerides (R = −0.23; $P = .02$), lower HDL cholesterol levels (R = 0.32; $P = .001$), and increased HOMA scores (R = −0.42; $P = .002$). Multivariate longitudinal analyses of all available measures of these patients found that lower HDL cholesterol levels and higher HOMA scores remained associated with both decreased leg fat (either normalized or kilograms) and increased BMI (in respective joint models of leg fat level [in kilograms] and BMI, the *P* values for HDL cholesterol were .002 and .001, and the *P* values for HOMA score were. 01 and <.001).

Effects of Nonthymidine NRTI Therapy: Initiating and Switching Therapy

There was no evidence of adipocyte mtDNA depletion among 15 patients initiating abacavir-based therapy or 2 patients initiating tenofovir-based therapy (median duration of exposure, 5.9 months; IQR, 5.3–6.5 months; $P = .8$, compared with treatment-naive participants), nor was there evidence of adipose tissue pathology (Figure 2). To consider nonthymidine NRTI treatment in the context of NRTI switching strategies, 39 biopsy

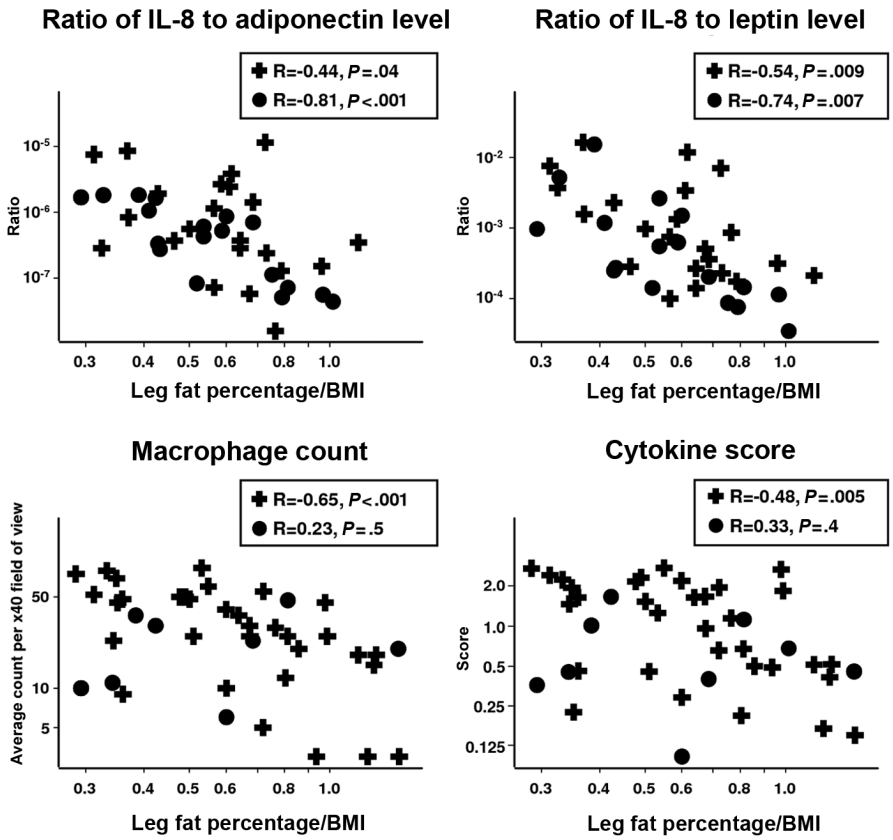

Figure 3. Relationship of lipoatrophy with markers of inflammation in tissue of thymidine-experienced patients. Spearman correlations are noted for patients who were currently receiving a thymidine-based regimen (+) or switched to an abacavir- or tenofovir-based regimen (●). BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); IL-8, interleukin-8.

samples were obtained from patients who had previously received thymidine-based NRTI treatment (33 abacavir recipients and 8 tenofovir recipients). Severity of lipoatrophy among these post-switch patients correlated with cumulative prior thymidine exposure ($R = -0.43$; $P < .001$) rather than total duration of therapy ($R = -0.34$; $P = .04$), and it remained strongly associated with ratio of IL-8 to adiponectin level ($R = -0.81$; $P < .001$) and ratio of IL-8 to leptin level ($R = -0.74$; $P = .007$) but not with macrophage count or proinflammatory cytokine scores.

Among 21 individuals who provided biopsy samples both before and after switching to nonthymidine NRTI therapy (median duration since switching, 7.0 months; IQR, 5.7–8.5 months), NRTI switching had no detectable effect on leg fat level over the time periods considered ($P = .9$). However, post-switch mtDNA levels were significantly higher than values during thymidine therapy ($P = .01$). Decreases in macrophage count and proinflammatory cytokine scores correlated with higher baseline values (data for 7 and 8 patients, respectively; $P = .04$ and .003, respectively), whereas decreases in the ratio of IL-8 to adiponectin level were associated with both higher preswitch cytokine values and less severe leg fat reduction before the switch (data for 16 patients; $P = .006$ and .04, respectively, in a joint model).

DISCUSSION

This study used a large number of adipose biopsies and body composition assessments from Western Australian HIV Cohort participants that were collected during the period 2001–2007, during which zidovudine- and stavudine-based treatment regimens were progressively phased out of clinical practice. With respect to this study, this has provided the opportunity to observe the long-term effects of ART choice on body composition and metabolic outcomes.

Our findings indicate that adipose tissue pathological changes and consequent lipoatrophic fat loss are highly prevalent among patients treated with thymidine NRTI-based treatment regimens. Among adult men with >12 months of exposure to these drugs, we found that one-half of zidovudine recipients and two-thirds of those treated with stavudine had leg fat measures less than the lowest decile value derived from untreated HIV-infected control subjects. In keeping with previous studies, we found that lipoatrophy is associated with—and preceded by—adipocyte mi-

tochondrial DNA depletion [10–12, 25, 27–29] and adipose tissue inflammation accompanied by proinflammatory adipocytokine changes [12, 13, 30, 31]. The morphological characteristics of this adipose tissue pathology are consistent with the "crown-like structures" observed in white adipose tissue samples obtained from obese humans and animals, in which activated macrophages form syncytia around necrotic adipocytes [32]. These are reproduced abundantly in lipoatrophic tissue specimens [25, 27], implying a common pathological pathway by which these conditions may associate with chronic adipose tissue inflammation and the "metabolic syndrome" phenotype. This is in keeping with findings in this and other studies that lipoatrophy (preferential subcutaneous fat loss) and increased BMI (overall adiposity) both contribute to risk of insulin resistance and elevated ratio of total to HDL cholesterol [7, 24, 33].

The influence of adipose tissue mitochondrial toxicity on whole-body metabolism is of interest in this context, given that the predominant role of adipose tissue is to store (rather than use) energy resources. A previous study involving healthy subjects [34] revealed striking correlations between adipocyte mtDNA copy number and adipose lipogenic activity over a range of mitochondrial DNA values entirely concordant with HIV-infected control subjects in this study. It is therefore plausible that pathological adipocyte mtDNA depletion associated with thymidine NRTI treatment induces not only widespread cell death and loss of fat mass, but also persistent defects in adipose tissue function that increase exposure of the liver and skeletal muscle to fatty acids [35, 36]. This loss of adipose tissue "fatty acid trapping" thus contributes to insulin resistance and dyslipidemia [37, 38], even before fat loss can be demonstrated [28, 39]. This view of lipoatrophy as an adipose-specific mitochondrial toxicity associated with both lethal and sublethal cellular injury and loss of adipose endocrine function is consistent with a model of disease pathogenesis that was initially proposed in 2001 [40].

These findings indicate that persistent adipose tissue pathology needs to be considered as a risk factor for metabolic complications. This issue has been addressed in recent guidelines for the prevention and management of metabolic diseases in HIV-infected persons [41] that recommend regular monitoring of fasting metabolic status and preemptive switching of thymidine NRTI–based therapy when possible. This policy is supported by our data indicating that the benefits of NRTI switching appear to be greatest in those with evidence of active adipose tissue inflammation (highly suggestive of ongoing adipocyte cell death [32]), but who have not progressed to more severe fat loss. In a broader sense, recognition of a prominent role for adipocyte death [32, 42] and abnormal adipose tissue morphology and function [43] in the pathogenesis of obesity-associated metabolic complications also provides a basis for the development of treatment strategies that may be beneficial for those affected by lipoatrophy. In this context, recent evidence that adipocyte turnover continues throughout adult life and is more dynamic than previously considered [43] provides hope that the restoration of adipose tissue metabolic and cytokine balance may also promote adipose tissue regeneration over time.

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Potential conflicts of interest. All authors: no conflicts.

References

1. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS **1998**; 12:F51–F58.
2. John M, Nolan D, Mallal S. Antiretroviral therapy and the lipodystrophy syndrome. Antivir Ther **2001**; 6:9–20.
3. Saint-Marc T, Partisani M, Poizot-Martin I, et al. Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: preliminary results of the LIPOCO study. AIDS **2000**; 14:37–49.
4. Mallal SA, John M, Moore CB, et al. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. AIDS **2000**; 14:1309–1316.
5. Gallant JE, Staszewski S, Pozniak AL, et al; 903 Study Group. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA **2004**; 292: 191–201.
6. Podzamczer D, Ferrer E, Sanchez P, et al; ABCDE (Abacavir vs. d4T (stavudine) plus efavirenz) Study Team. Less lipoatrophy and better lipid profile with abacavir as compared to stavudine: 96-week results of a randomized study. J Acquir Immune Defic Syndr **2007**; 44:139–147.
7. Kosmiski LA, Bacchetti P, Kotler DP, et al. Relationship of fat distribution with adipokines in human immunodeficiency virus infection. J Clin Endocrinol Metab **2008**; 93:216–224.
8. Young J, Rickenbach M, Weber R, et al. Body fat changes among antiretroviral-naive patients on PI- and NNRTI-based HAART in the Swiss HIV cohort study. Antivir Ther **2005**; 10:73–81.
9. van Vonderen MG, van Agtmael MA, Hassink EA, et al; MEDICLAS study group. Zidovudine/lamivudine for HIV-1 infection contributes to limb fat loss. PLoS One **2009**; 4:e5647.
10. Cherry CL, Nolan D, James IR, et al. Tissue-specific associations between mitochondrial DNA levels and current treatment status in HIV-infected individuals. J Acquir Immune Defic Syndr **2006**; 42:435–440.
11. Nolan D, Hammond E, James I, et al. Contribution of nucleoside-analogue reverse transcriptase inhibitor therapy to lipoatrophy from the population to the cellular level. Antivir Ther **2003**; 8:617–626.
12. Sievers M, Walker UA, Sevastianova K, et al. Gene expression and immunohistochemistry in adipose tissue of HIV type 1–infected patients with nucleoside analogue reverse-transcriptase inhibitor-associated lipoatrophy. J Infect Dis **2009**; 200:252–262.
13. Jones SP, Qazi N, Morelese J, et al. Assessment of adipokine expression and mitochondrial toxicity in HIV patients with lipoatrophy on stavudine- and zidovudine-containing regimens. J Acquir Immune Defic Syndr **2005**; 40:565–572.
14. Barragan P, Fisac C, Podzamczer D. Switching strategies to improve lipid profile and morphologic changes. AIDS Rev **2006**; 8:191–203.
15. Tebas P, Zhang J, Hafner R, et al. Peripheral and visceral fat changes following a treatment switch to a non-thymidine analogue or a nu-

cleoside-sparing regimen in HIV-infected subjects with peripheral lipoatrophy: results of ACTG A5110. J Antimicrob Chemother **2009**; 63:998–1005.
16. D:A:D Study Group, Sabin CA, Worm SW, Weber R, et al. Use of nucleoside reverse transcriptase inhibitors and risk of myocardial infarction in HIV-infected patients enrolled in the D:A:D study: a multicohort collaboration. Lancet **2008**; 371:1417–1426.
17. Podzamczer D, Ferrer E, Martínez E, et al; ABCDE Study Team. How much fat loss is needed for lipoatrophy to become clinically evident? AIDS Res Hum Retroviruses **2009**; 25:563–567.
18. Rajagopalan R, Laitinen D, Dietz B. Impact of lipoatrophy on quality of life in HIV patients receiving anti-retroviral therapy. AIDS Care **2008**; 20:1197–1201.
19. Crane HM, Grunfeld C, Harrington RD, et al. Lipoatrophy among HIV-infected patients is associated with higher levels of depression than lipohypertrophy. HIV Med **2008**; 9:780–786.
20. Hammond E, Nolan D. Adipose tissue inflammation and altered adipokine and cytokine production in antiretroviral therapy-associated lipodystrophy. Curr Opin HIV AIDS **2007**; 2:274–281.
21. Frayn KN, Karpe F, Fielding BA, et al. Integrative physiology of human adipose tissue. Int J Obes Relat Metab Disord **2003**; 27:875–888.
22. De Socio GV, Parruti G, Quirino T, et al; CISAI study group. Identifying HIV patients with an unfavorable cardiovascular risk profile in the clinical practice: results from the SIMONE study. J Infect **2008**; 57:33–40.
23. De Wit S, Sabin CA, Weber R, et al; Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. Diabetes Care **2008**; 31:1224–1229.
24. Wohl D, Scherzer R, Heymsfield S, et al; FRAM Study Investigators. The associations of regional adipose tissue with lipid and lipoprotein levels in HIV-infected men. J Acquir Immune Defic Syndr **2008**; 48: 44–52.
25. Nolan D, Hammond E, Martin A, et al. Mitochondrial DNA depletion and morphologic changes in adipocytes associated with nucleoside reverse transcriptase inhibitor therapy. AIDS **2003**; 17:1329–1338.
26. Hammond EL, Sayer D, Nolan D, et al. Assessment of precision and concordance of quantitative mitochondrial DNA assays: a collaborative international quality assurance study. J Clin Virol **2003**; 27:97–110.
27. Walker UA, Bickel M, Lütke Volksbeck SI, et al. Evidence of nucleoside analogue reverse transcriptase inhibitor-associated genetic and structural defects of mitochondria in adipose tissue of HIV-infected patients. J Acquir Immune Defic Syndr **2002**; 29:117–121.
28. Boothby M, McGee KC, Tomlinson JW, et al. Adipocyte differentiation, mitochondrial gene expression and fat distribution: differences between zidovudine and tenofovir after 6 months. Antivir Ther **2009**; 14:1089–1100.
29. Buffet M, Schwarzinger M, Amellal B, et al. Mitochondrial DNA depletion in adipose tissue of HIV-infected patients with peripheral lipoatrophy. J Clin Virol **2005**; 33:60–64.
30. Kratz M, Purnell JQ, Breen PA, et al. Reduced adipogenic gene expression in thigh adipose tissue precedes human immunodeficiency virus–associated lipoatrophy. J Clin Endocrinol Metab **2008**; 93:959–966.
31. Kim MJ, Leclercq P, Lanoy E, et al. A 6-month interruption of antiretroviral therapy improves adipose tissue function in HIV-infected patients: the ANRS EP29 Lipostop Study. Antivir Ther **2007**; 12:1273–1283.
32. Cinti S, Mitchell G, Barbatelli G, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res **2005**; 46:2347–2355.
33. Estrada V, Martínez-Larrad MT, González-Sánchez JL, et al. Lipodystrophy and metabolic syndrome in HIV-infected patients treated with antiretroviral therapy. Metabolism **2006**; 55:940–945.
34. Kaaman M, Sparks LM, van Harmelen V, et al. Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue. Diabetologia **2007**; 50:2526–2533.
35. Sevastianova K, Sutinen J, Kannisto K, et al. Adipose tissue inflammation and liver fat in patients with highly active antiretroviral therapy-associated lipodystrophy. Am J Physiol Endocrinol Metab **2008**; 295: E85–E91.
36. Kosmiski LA, Ringham BM, Grunwald GK, et al. Dual-energy X-ray absorptiometry modeling to explain the increased resting energy expenditure associated with the HIV lipoatrophy syndrome. Am J Clin Nutr **2009**; 90:1525–1531.
37. van Wijk JP, Cabezas MC, de Koning EJ, et al. In vivo evidence of impaired peripheral fatty acid trapping in patients with human immunodeficiency virus-associated lipodystrophy. J Clin Endocrinol Metab **2005**; 90:3575–3582.
38. Ware LJ, Jackson AG, Wootton SA, et al. Antiretroviral therapy with or without protease inhibitors impairs postprandial TAG hydrolysis in HIV-infected men. Br J Nutr **2009**; 102:1038–1046.
39. Blümer RM, van Vonderen MG, Sutinen J, et al. Zidovudine/lamivudine contributes to insulin resistance within 3 months of starting combination antiretroviral therapy. AIDS **2008**; 22:227–236.
40. Nolan D, John M, Mallal S. Antiretoviral therapy and the lipodystrophy syndrome, part 2: concepts in aetiopathogenesis. Antivir Ther **2001**; 6:145–160.
41. Lundgren JD, Battegay M, Behrens G, et al; EACS Executive Committee. European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV. HIV Med **2008**; 9:72–81.
42. Alkhouri N, Gornicka A, Berk MP, et al. Adipocyte apoptosis:aA link between obesity, insulin resistance and hepatic steatosis. J Biol Chem **2009**; 285:3428–3438.
43. Arner E, Westermark PO, Spalding KL, et al. Adipocyte turnover: relevance to human adipose tissue morphology. Diabetes **2010**; 59:105–109.