In Vivo, Nucleoside Reverse-Transcriptase Inhibitors Alter Expression of Both Mitochondrial and Lipid Metabolism Genes in the Absence of Depletion of Mitochondrial DNA

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Background. Nucleoside reverse-transcriptase inhibitors (NRTIs), which are used to treat human immunodeficiency virus (HIV) infection, can cause mitochondrial dysfunction and have been associated with lipoatrophy. The effects of this mitochondrial dysfunction on lipid metabolism, at a molecular level in vivo, have not been described.

Methods. We examined early changes (by 2 weeks after initiation of therapy) in expression of mitochondrial and nuclear genes in adipose tissue from 20 HIV-negative subjects randomized to receive dual-NRTI therapy (zidovudine/lamivudine or stavudine/lamivudine) for 6 weeks.

Results. We observed decreased transcription of mitochondrial (mt) RNA without significant depletion of mtDNA. Decreases in mtRNA coincided with simultaneous up-regulation of nuclear genes involved in transcriptional regulation of mtRNA (NRF1 and TFAM) and oxidation of fatty acids (PPARA and LPL), whereas PPARG, which is important for differentiation of adipose tissue, was down-regulated. Many nuclear changes correlated with changes in peroxisome proliferator–activated receptor—γ coactivator—1 (PGC1), suggesting a central role for PGC1 in nuclear responses to mitochondrial dysfunction. Expression of peripheral blood monocyte mtRNA also decreased, suggesting that monocytes may be surrogates for NRTI-induced mitochondrial dysfunction in other tissues.

Conclusions. Independent of HIV, NRTIs decrease transcription of mtRNA in vivo. The absence of depletion of mtDNA suggests that NRTIs cause mitochondrial dysfunction by means other than through inhibition of DNA polymerase—γ, whereas disruption of expression of lipid metabolism genes offers an explanation for NRTI-induced lipoatrophy.

Combinations of 2 nucleoside reverse-transcriptase inhibitors (NRTIs) form the backbone of antiretroviral therapy (ART) for treatment of HIV infection [1]. Most NRTIs, including the thymidine analogues zidovudine and stavudine, can induce toxicities such as lipoatrophy, peripheral neuropathy, liver steatosis, myopathy, and...
perinatal encephalopathy [2–8]. Approximately 50% of HIV-infected adults treated with ART develop HIV-associated lipodystrophy (HIVLD), which is characterized by selective subcutaneous (sc) lipoatrophy, relative gain of central fat, dyslipidemia, and insulin resistance [2–3, 9]. These metabolic complications are associated with increased incidence of myocardial infarction [10].

Both HIV protease inhibitors (PIs) and NRTIs contribute to HIVLD [11]—NRTIs possibly by affecting mitochondrial function and PIs by inhibiting sterol regulatory element–binding protein–1 (SREBP1) and peroxisome proliferator–activated receptor (PPAR) γ [12–14]. SREBP1 and PPARγ, together with PPARα, regulate transcription of lipid metabolism genes. This regulation is linked to mitochondrial physiology through PPARγ coactivator 1 (PGC1). PGC1 not only interacts with PPARα and PPARγ to affect transcription of lipid metabolism genes [15–16] but also regulates transcription of mitochondrial genes through nuclear respiratory factor (NRF) 1 and 2, which regulate mitochondrial transcription factor A (mtTFA) [16–18] (figure 1).

Through these mechanisms, PGC1 can influence production of ATP in cells that use lipid as an energy substrate [15–17, 19]. Although changes in expression of PGC1 can affect transcription of mtRNA, it is unclear whether the reverse is true. In particular, mechanisms whereby NRTI-induced mitochondrial toxicity affects lipid metabolism, leading to lipoatrophy, are poorly understood.

NRTIs undergo intracellular and intramitochondrial phosphorylation to active triphosphates capable of inhibiting HIV reverse transcriptase (RT). In vitro, some NRTIs inhibit DNA polymerase-γ (DNA pol-γ), a nuclear-encoded polymerase important for replication of mtDNA [20]. Since mitochondria are central to cellular energy production through oxidative phosphorylation (OXPHOS) and the mitochondrial electron transport chain (mtETC), it is thought that depletion of mtDNA, induced by inhibition of DNA pol-γ, may decrease cellular OXPHOS capacity [21]. Although direct inhibition of DNA pol-γ by NRTIs has not been demonstrated in vivo, mtDNA quantity is commonly used as a surrogate for NRTI toxicity [4].

The mtETC comprises 5 complexes (I–V) encoded by both nuclear and mitochondrial genes. Mitochondrial-encoded subunits—including cytochrome b (Cyt b) of complex III and cytochrome c oxidase subunits I, II, and III (COX1, -2, and -3) of complex IV—play important functional roles [22], whereas nuclear-encoded components, such as COX4, play roles that are more regulatory than functional [18]. Through a process called “mitochondrial fine tuning,” mitochondria can adapt transcription of mtRNA to meet altered OXPHOS requirements independent of regulation by nuclear factors or changes in mtDNA level [18, 23]. To test the hypothesis that NRTI-induced mitochondrial dysfunction affects lipid metabolism, we examined the early effects of commonly used NRTI combinations on adipose tissue expression of genes and mtDNA in HIV-negative subjects.

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**Figure 1.** Regulatory pathways involved in expression of mitochondrial and lipid metabolism genes. COX, cytochrome c oxidase; Cyt b, cytochrome b; FAO, fatty acid oxidation; FAs, fatty acids; LPL, lipoprotein lipase; mtTFA, mitochondrial transcription factor α; NRF1, nuclear respiratory factor 1; PPAR, peroxisome proliferator–activated receptor; PGC1, PPARγ coactivator 1; SREBP1, sterol regulatory element–binding protein 1.
SUBJECTS, MATERIALS, AND METHODS

Population and study design. Subjects (age >18 years) were recruited through St. Vincent’s Hospital, Sydney. Exclusion criteria included pregnancy; HIV infection; history of diabetes, coagulopathy, or another serious illness; active hepatitis B infection; recent use (within the previous 6 months) of sex hormones; use of oral glucocorticoid or anabolic steroids; and any prior exposure to ART. Subjects were randomized (1:1) to receive either stavudine (40 mg twice daily) and lamivudine (150 mg twice daily) or zidovudine (300 mg twice daily) and lamivudine (150 mg twice daily) for 6 weeks. The study was approved by St. Vincent’s Hospital Human Research Ethics Committee, and subjects provided written, informed consent before enrollment.

Clinical assessments. Subjects attended the clinic at weeks 0, 1, 2, 3, 6, and 12, at which times clinical assessment and safety blood analyses (full blood count and determination of electrolytes, liver enzymes, urea, and creatinine) were performed. Fasting metabolic and body composition (3-slice abdominal computed tomography and dual-energy x-ray absorptiometry) indices were assessed as described elsewhere [24].

Sample processing. At weeks 0 and 2, we performed sc fat biopsies under local anesthetic on fasted subjects by use of a true-cut incision in the flank and removal of up to 2 g of adipose tissue. Week 2 was chosen as the point at which to determine early drug-induced effects, to avoid potential confounding effects of changes in diet or exercise arising over time. We processed samples immediately, by fixing one 5-mm sample in 10% phosphate-buffered formalin, for histological analysis, and two 1-mm samples in 0.1 mol/L phosphate-buffered 2.5% glutaraldehyde (pH 7.4) with 1 mmol/L calcium chloride, for electron microscopic analysis. After overnight fixation, formalin-fixed samples were processed to paraffin, sectioned at 4 µm, and stained with hematoxylin-eosin before light microscopic analysis (×10 objective) and digital photography by use of a Zeiss AxioCam with AxioVision software. An observer, blinded to the treatment arms, determined the number of adipocytes per photographed high magnification (×21,000 magnification) by use of a Philips 1410 electron microscope (Philips Electronics). Remaining tissue was separated into aliquots (300 mg).

We examined monocytes as peripheral blood surrogates for NRTI-induced mitochondrial toxicity. The choice to use them as surrogates was based on certain characteristics, including the importance of lipid metabolism genes in the regulation of monocyte cholesterol metabolism [25] and the high expression of mtRNA (noted in preliminary experiments) in these cells. Collections at weeks 0, 6, and 12 were chosen to coincide with other routine study analyses. We isolated monocytes from whole blood–derived buffy coats by positive selection using anti-CD14–conjugated magnetic beads (Dynal Biotech), in accordance with the manufacturer’s instructions. This yielded (mean ± SD) 2.4 (± 1.5) × 10⁶ cells/collection, with 80% depletion of monocytes from whole blood and <10% contamination of lymphocytes. Aliquots of fat and monocytes were suspended in Trizol (1 mL; Invitrogen Life Technologies) and stored at −70°C immediately after collection.

Primer selection. We designed primers, by use of Primer3 Web-based software (available at: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), from gene sequences located in the Enterz Nucleotides database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide&tool=toolbar) (Table 1) and diluted them to 50 µmol/L for polymerase chain reactions (PCRs). To enable detection of gDNA contamination, we designed β-actin primers across an intron, giving 2 product sizes; 116 bp for first-strand cDNA and 211 bp for gDNA. Since we aimed to examine effects on transcription of mtRNA throughout the full extent of the mitochondrial H-loop, in addition to examining changes in genes that would have important functional consequences, we chose to examine 3 functionally important mitochondrial mRNAs (MTCO1 [the gene for COX1], MTCO3 [the gene for COX3], and MTCYB [the gene for Cyt b]), since these genes are scattered throughout the H-loop of the mitochondrial genome [18].

RNA and DNA isolation. We extracted RNA from homogenized fat and monocytes by use of the Trizol extraction protocol (Invitrogen), followed by DNase reaction (RQ1 DNase; Promega) and RNA cleanup (RNeasy Mini Kit; Qiagen). We quantified RNA by use of a spectrophotometer with SYBR Green II (Boehringer Mannheim) and RNA cleanup (QIAamp DNA Blood Mini Kit; Qiagen). We quantified DNA from homogenized skeletal muscle by use of a spectrophotometer with SYBR Green II (Molecular Probes) against known standard RNA concentrations and determined quality by examination of 18S and 28S RNA on a 1.5% agarose gel. This yielded (mean ± SD) 3.05 (± 1.84) µg of total RNA/100 mg of adipose tissue and 2.83 (± 2.02) µg of total RNA/monocyte sample. From the remaining homogenate we extracted DNA by use of the Trizol protocol, followed by DNA cleanup (QIAamp DNA Blood Mini Kit; Qiagen) and quantification by use of SYBR Green I (Boehringer Mannheim) against known standard DNA concentrations.

RT reaction and PCR. We prepared cDNA from RNA samples (200 ng) by use of SuperscriptII RT and oligo dT primers (Invitrogen), to a final volume of 20 µL. To adjust for inter-RT variability, we performed 4 RT reactions/sample and pooled the cDNA. We used 2-µL (20-ng) aliquots of cDNA or gDNA for real-time quantitative PCR, by use of the Lightcycler platform with Hot Start reaction mix (Roche Applied Science), in

Table 1. Gene profiles.

The table is available in its entirety in the online edition of the Journal of Infectious Diseases.
in accordance with the manufacturer’s instructions. Samples were run in duplicate, with internal positive and negative controls, and gene product was quantified by comparing samples to known standard concentrations of pure gene product. Overall, the mean (± SD) intrasample variability coefficient of variation (cv) was 7% (± 4%), and the mean ± SD intersubject cv was 72% (± 18%).

Expression of several genes was >2 log₂ lower than that of the house-keeping gene ACTB. Therefore, detection of these genes required samples to have expression of ACTB >1.5 × 10⁻⁶ ng/μL. One of 40 fat samples had expression of ACTB (the gene for β-actin) below the required sensitivity and was omitted from further analysis. Two samples yielded RNA sufficient to measure 8 and 11 of the 13 genes analyzed, respectively. Expression of ACTB did not change between baseline and week 2, and there were no between-group differences at either time point. Results were presented, for genes, as the ratio of expression of the gene to expression of ACTB. For mtDNA, we compared expression of a mitochondrial gene (MTCYB), for which results were expressed as nanograms, with that of a nuclear gene (PPARG [the gene for PPARγ]), for which results were expressed as copy number (2 copies/cell), with final results expressed as nanograms per cell.

Statistical analysis. Unless stated, results are presented as medians and interquartile ranges (IQRs). We used the Wilcoxon signed rank test for comparisons of differences between groups of variables, the Mann-Whitney U test for between-group differences, and Spearman’s rank correlation (r) for correlations between variables, with P < .05 considered to be significant. We did not adjust P values for multiple comparisons.

RESULTS

All 20 HIV-negative subjects were white (mean [± SD] age, 41 [± 15] years), and 90% were male. We recorded 29 adverse events, the most common being headache and fatigue. One subject discontinued zidovudine/lamivudine at week 4 because of persistent nausea but continued with follow-up. All 20 subjects completed the 12 weeks of the study and undertook all study procedures.

Adipose tissue expression of mtRNA and mtDNA. Adipose tissue expression of 3 mtRNAs (MTCO1, MTCO3, and MTCYB) decreased significantly by week 2 (figure 2A). The changes in expression of all 3 genes were significantly correlated (ρ = 0.49–0.72; P = .04–.002). Expression of nuclear-encoded COX4I2 (the gene for COX4) did not change (figure 2D). Although individual mtDNA levels at baseline correlated with those at week 2 (ρ = 0.65; P = .008), there was no significant change in mtDNA levels between baseline and week 2 (85 vs. 75 ng/cell; P = .12; median percentage change, +16% [IQR, 94%]) (figure 3A), suggesting that the decreases in expression of mtRNA were not secondary to depletion of mtDNA.

Adipose tissue expression of nuclear genes. Expression of PPARGC1A (the gene for PGC1), NRF1 (the gene for NRF1), and TFAM (the gene for mtTFA) increased significantly at week 2 (all P ≤ .02) (figure 2B). Changes in expression of PPARGC1A correlated with those of NRF1 (ρ = 0.6; P = .001) but not with those of TFAM (ρ = 0.38; P = .1), and changes in expression of NRF1 correlated with those of TFAM (ρ = 0.63; P = .009), supporting a regulatory role for PPARGC1A in expression of NRF1 and for NRF1 in expression of TFAM. However, that changes in expression of TFAM did not correlate with those of mtRNA suggests that changes in expression of TFAM were not responsible for the observed changes in expression of mtRNA.

Expression of PPARG decreased by 50% (P = .003) (figure 2C). Changes in expression of PPARG correlated with changes in expression of MTCO1, MTCO3, and MTCYB (ρ = 0.49–0.85; P = .04–.0003) but not with changes in expression of other transcription factors (SREBF1 [the gene for SREBP1], ρ = 0.02; PPARGC1A, ρ = 0.06; PPARA [the gene for PPARα], ρ = 0.18). Expression of PPARA increased significantly (figure 2C), but changes in expression of PPARG did not correlate with those of any other measured gene. Expression of SREBF1 did not change.

Expression of LPL (the gene for lipoprotein lipase [LPL]), which is involved in recruitment of circulating lipid substrate for OXPHOS [26], increased significantly (figure 2D). Values for PPARGC1A correlated with those for LPL at baseline (ρ = 0.68; P = .005) and at week 2 (ρ = 0.85; P = .0003), as did changes in expression of LPL with those of PPARGC1A (ρ = 0.7; P = .004), suggesting a regulatory role for PPARGC1A in expression of LPL.

Expression of LEP (the gene for leptin) significantly increased (figure 2D). Changes in expression of LEP correlated positively with those of PPARGC1A (ρ = 0.64; P = .008) and negatively with those of mtRNA (MTCYB: ρ = –0.49, P = .05; MTCO1: ρ = –0.63, P = .01). There were no correlations between leptin mRNA and serum leptin concentrations at either baseline or week 2 (P > .1), although serum leptin concentrations correlated closely with limb fat mass at all time points.

Expression of monocyte mtRNA. Expression of monocyte MTCO1 decreased significantly at week 6, and this change persisted to week 12, 6 weeks after discontinuation of treatment (figure 3C). A follow-up sample of monocytes, collected a median of 66 weeks after baseline [27], showed that expression of MTCO1 returned to baseline levels. Although baseline expression of MTCO1 in monocytes correlated with MTCO1 in fat (ρ = 0.66; P = .01), there was no significant correlation between the changes in MTCO1 expression at week 2 in adipose tissue and the changes in MTCO1 expression at week 6 in monocytes (ρ = –0.02), perhaps because samples were collected at different time points.

Between-group differences. Use of stavudine-containing
regimens has been associated with greater lipodystrophy [3] and depletion of mtDNA [7]. Although we observed larger decreases in adipose tissue expression of $MTCO1$ and $MTCYB$ in the stavudine/lamivudine group than in the zidovudine/lamivudine group (median [IQR], 81% [77%] vs. 44% [76%] and 71% [56%] vs. 43% [79%], respectively), these differences were not statistically significant ($P = .1$). In addition, there were no between-group differences in adipose tissue expression of $MTCO3$, 

**Figure 2.** Changes in adipose tissue expression of genes 2 weeks after exposure to nucleoside reverse-transcriptase inhibitors (NRTIs). Shown is expression before (■) and 2 weeks after (□) NRTI treatment. **A,** Expression of mitochondrial genes; **B,** nuclear genes involved in the regulation of mitochondrial transcription; **C,** nuclear transcription factors; **D,** downstream genes involved in lipid metabolism and the mitochondrial electron transport chain. The nos. in panels represent the median (interquartile range) percentage change from baseline; statistical significance was determined by use of the Wilcoxon signed rank test.
Figure 3. Adipose tissue expression of mtDNA, β-actin mRNA, and monocyte cytochrome c oxidase 1 (COX1) mRNA. Shown is expression before (open bars) and 2 weeks after (closed bars) nucleoside reverse-transcriptase inhibitor treatment. A, Adipose tissue expression of mtDNA; B, expression of β-actin mRNA; C, expression of mitochondrial genes in monocytes. The nos. represent the median (interquartile range) percentage change from baseline; statistical significance was determined by use of the Wilcoxon signed rank test.

mtDNA, or monocyte COX1. Apart from PPARA, which was up-regulated more in the stavudine/lamivudine group (median [IQR], 115% [87%]) than in the zidovudine/lamivudine group (median [IQR], 17% [75%]) (P < .03), there were no other between-group differences noted in expression of nuclear genes.

Body composition and adipose tissue structure. Body composition did not change (table 2). By light microscopy, adipocyte size at baseline varied considerably between individuals (figure 4A), although adipocyte number did not change (median [IQR], 62 [23] and 65 [29] cells/high-powered field at baseline and week 2, respectively; P = .6), and no inflammatory or lipogranulomatous changes, changes in mitochondrial morphology, or intramitochondrial inclusions, granules, or giant mitochondria were observed (figure 4B). With the exception of serum leptin—which, after a small increase at week 2 (median increase, 4–4.7 mmol/L; P = .17), decreased significantly at week 6 and returned to baseline levels at week 12—there were no changes in clinical metabolic indices and no unexpected changes in other clinical parameters (table 2).

DISCUSSION

The present study is, to our knowledge, the first prospective in vivo molecular examination of the effect of NRTI-induced mitochondrial dysfunction on lipid metabolism. Exposure to 2 commonly used NRTI combinations for only 2 weeks significantly reduced adipose tissue expression of mtRNA (without significant depletion of mtDNA) and of nuclear genes involved in several aspects of lipid metabolism. The observed reductions in expression of mtRNA are likely to be physiologically significant, since cellular OXPHOS capacity is closely linked to expression of mtRNA [27]. The nuclear responses, in particular the decreased expression of PPARA, may explain how long-term exposure to NRTIs contributes to lipoatrophy.
Since NRTI-induced mitochondrial dysfunction, mediated by inhibition of DNA pol-γ [20], has not been demonstrated in vivo, examination of tissue and blood mtDNA levels has yielded conflicting results [7, 27–29], and evidence of decreased OXPHOS capacity, induced by NRTIs, has been observed without changes in mtDNA levels [5]. Despite this, mtDNA level remains the standard surrogate measurement for this toxicity. We found no evidence that depletion of mtDNA is a potential cause of the observed early decreases in expression of mtRNA. However, since we used primers for mtDNA analysis that were located within \( MTCYB \), close to the replication initiation site of the H-strand of mtDNA [18], we are unable to comment on the quality of full-length mtDNA. Further prospective studies are needed to assess changes in mtDNA quality with therapy. Nevertheless, that expression of \( MTCYB \) decreased to a similar extent as did that of \( MTCO1 \) and \( MTCO3 \) supports a general inhibition of mitochondrial transcription rather than any change in mtDNA length resulting from NRTI-induced chain termination.

Furthermore, whereas normal expression of mtRNA is vital for replication of mtDNA [18], the reverse is not true. Cells depleted of mtDNA maintain expression of mtRNA, and depletion of mtDNA alone is insufficient to induce up-regulation of nuclear-encoded mtDNA replication factors [18, 30–31].
Figure 4. Light and electron microscopic analysis of adipose tissue before and 2 weeks after exposure to nucleoside reverse-transcriptase inhibitors (NRTIs). A, High-power field (×10) images of adipose tissue biopsy specimens from 2 patients before and after exposure to NRTIs. B, Electron microscopic analysis of adipocytes from a patient before and 2 weeks after exposure to NRTIs.
However, it is possible that persistent NRTI-induced down-regulation of transcription of mtRNA would affect replication of mtDNA and ultimately lead to the depletion of mtDNA seen in adipose tissue biopsy specimens from HIV-positive subjects who have received long-term ART [7].

Our data also offer valuable insights into the nuclear responses to mitochondrial dysfunction. In vitro, expression of PPARGC1A is up-regulated in response to external stresses, such as exposure to cold [16] and starvation [19], resulting in increased OXPHOS capacity through up-regulation of genes involved in the mtETC and fatty acid oxidation (FAO). We propose that, in vivo, decreased OXPHOS capacity results from NRTI-induced reductions in mtRNA, causing up-regulation of PPARGC1A and other nuclear genes in an attempt to compensate. Although feedback pathways between mitochondria and the nucleus in yeast have been proposed [32], evidence to support the existence of such retrograde pathways in humans has not previously been reported.

The results of the present study are consistent with previous findings that PPARGC1A coordinates up-regulation of regulatory pathways aimed at stimulating mitochondrial transcription (NRF1 and TFAM) [15], FAO (PPARA) [14], and triglyceride availability (LPL) [26]. That these genes are up-regulated and, in the case of mtTFA, that they fail to stimulate transcription of mitochondrial genes suggest that the basis of the drug toxicity is within the mitochondria itself rather than within the nucleus.

The observed increases in expression of PPARGC1A seem to contradict the results of a previous study that showed decreased expression of PPARGC1A in adipose tissue from HIV-infected subjects with lipodystrophy [14]. The latter study was, however, a cross-sectional analysis of adipose tissue from patients with and without lipodystrophy who had been treated with a variety of antiretroviral regimens, and, so, direct comparison is difficult.

If inhibition of mtRNA is not secondary to depletion of mtDNA, then how do NRTIs cause this effect? In vitro intramitochondrial accumulation of ATP and, paradoxically, ADP above certain concentrations inhibits transcription of mtRNA [18]. We propose that, through mitochondrial fine tuning, mitochondria could respond to intramitochondrial accumulation of phosphorylated NRTIs in the same way that they respond to elevations in ADP and ATP—that is, by down-regulating transcription of mtRNA independent of nuclear stimulation. Correlating intramitochondrial concentrations of phosphorylated NRTIs, after exposure to NRTIs, with transcription of mtRNA would help to test this hypothesis, although such experiments were beyond the scope of the present study.

Down-regulation of PPARG might appear to be contradictory, given the up-regulation of its coactivator, PPARGC1A. Although little has been published with regard to the PPARG/PPARGC1A relationship in vivo, overexpression of PPARGC1A in 3T3-L1 adipocytes also resulted in down-regulation of PPARG [15], providing a potential mechanism whereby long-term NRTI-induced mitochondrial dysfunction could induce lipodystrophy. Similar decreases have been noted in vitro in cells exposed to PIs [12], and low expression of PPARG is consistently found in fat biopsy specimens from adults with lipodystrophy [13, 14]. NRTI-induced decreases in expression of PPARG were not mediated through SREBF1, a target of PI toxicity, since expression of SREBF1 was unaffected. However, cumulative effects on PPARG by NRTIs and PIs could explain the increased risk of HIVLD with concurrent NRTI-PI therapy [11]. In addition, persistent NRTI-induced down-regulation of PPARG may explain why, in subjects with HIVLD, 48 weeks of treatment with rosiglitazone, a PPARG agonist, failed to increase limb fat mass in the setting of continued NRTI treatment [24].

Reductions in expression of monocyte COX1 show that NRTI-induced effects on transcription of mtRNA are not restricted to adipose tissue. However, the week-6 changes in monocytes were less than and did not correlate with the week-2 changes in fat. Further studies are required to determine the potential of monocytes as surrogate markers for NRTI-induced effects in adipose tissue.

The effects in monocytes persisted for at least 6 weeks after discontinuation of treatment. Given the relatively short half-life of circulating monocytes, the results suggest that these effects occur within the bone marrow rather than only within peripheral blood. Evidence of persistence of mitochondrial dysfunction long after exposure in children who were exposed to NRTIs in utero has been described [5]. Of note, in these children, defects in mitochondrial function were observed without significant depletion of mtDNA.

Although both serum leptin concentrations and adipose tissue expression of leptin genes increased at week 2, there were no correlations between changes in expression of LEP and serum leptin concentrations. Although expression of leptin is known to increase with a decrease in expression of PPARG [33], it may be that expression of leptin mRNA does not reflect serum leptin concentrations, since numerous factors—including fat mass (as demonstrated in the present study), glucocorticoid responsiveness, and sympathetic nervous activity—are known to affect serum leptin concentrations [33, 34].

Both NRTI combinations affected transcription of mtRNA. Although the present study was not designed to detect subtle between-group differences, the apparently greater changes in the stavudine/lamivudine group reflect the more-frequent and -severe lipodystrophy observed in patients treated with stavudine [4, 9]. However, this trend was not observed in all adipocyte genes and was not observed in monocytes.

All ex vivo studies have problems with potential contamination of samples with unwanted cell types. Our protocols minimized such contamination, and, by sequential sample analysis, any such
errors would have been spread equally across both time points and would have been unlikely to significantly affect the overall results. Analysis of further downstream target genes of transcription factors involved in both glucose and lipid metabolism would shed further light on the cellular consequences of mitochondrial toxicity.

In addition, although we have shown effects in 2 tissues, ART-induced abnormalities in lipid and mitochondrial metabolism in other tissues (such as the liver [35] and muscles [36, 37]) have been described, and it is not possible to extrapolate our results to other tissues. Although these results offer insights into the pathogenesis of NRTI-induced mitochondrial dysfunction and the potential of screening for mitochondrial dysfunction before the development of lipodystrophy, validation of these results in longitudinal cohorts of HIV-infected patients treated with a variety of antiretroviral drugs is required to confirm the link between inhibition of transcription of mtRNA in fat or monocytes and subsequent lipoatrophy. However, these data do suggest the potential for early screening for mitochondrial toxicity, which would be invaluable in the assessment of new antiretroviral drugs and would enable development of safer long-term treatments.

**ADDITIONAL SAMA INVESTIGATORS**

Additional SAMA Investigators are as follows: Michael Feneley and Jason Kovacic (Department of Cardiology, St Vincent’s Hospital, Sydney); Julie Miller, Kate Merlin, Mee Ling Munier, and Megan Winterbotham (HIV Immunovirology Laboratory, Centre for Immunology, St Vincent’s Research Campus); and Hugh Brierley (Department of Anatomical Pathology, St Vincent’s Hospital, Sydney).

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**References**

11. an der Valk M, Gisolf EH, Wit FWNM, Japour A, Wellerling GJ, Danner SA, on behalf of the Promethus study group. Increased risk of lipodystrophy when nucleoside analogue reverse transcriptase inhibitors are included with protease inhibitors in the treatment of HIV-1 infection. AIDS 2001; 15:847–55.
25. Moore KJ, Rosen ED, Fitzgerald ML, et al. The role of PPAR-γ in...


