Original article

Evidence for a shift to anaerobic metabolism in adipose tissue in efavirenz-containing regimens for HIV with different nucleoside backbones

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Background: Antiretroviral (ARV) treatment has been associated with abnormalities in lipid and mitochondrial metabolism. We compared patterns of gene expression in the subcutaneous adipose tissue (SAT) of HIV-positive subjects before and after 18–24 months of ARV therapy with HIV-negative controls.

Methods: HIV patients naive to ARV were randomized to receive zidovudine (AZT), lamivudine (3TC) with efavirenz (EFV) or tenofovir disoproxil fumarate (TDF) with emtricitabine (FTC) and EFV. Healthy controls (n=15) were matched for age, ethnicity and gender. Patients on a regimen containing abacavir (ABC), 3TC and EFV for 18–24 months were also tested. Genes involved in adipocyte glucocorticoid, lipid and mitochondrial metabolism, and adipocyte differentiation, were profiled with real-time PCR.

Results: AZT led to increased visceral adipose tissue (VAT; P=0.012) and VAT:SAT ratio (P=0.036), whereas TDF increased SAT (P=0.047) and peripheral fat/lean body mass ratio (P=0.017). HIV treatment-naive patients had lower plasma lipoprotein lipase (LPL) activity (P=0.0001) versus controls (remaining below controls after ARV; P=0.038–0.0001). The overall pattern of gene expression was similar across all treatment groups, being most marked with AZT and least with TDF. There was up-regulation of peroxisome proliferator-activated receptor-γ coactivator-1α, uncoupling protein-2 and hexose 6-phosphate dehydrogenase, and down-regulation of nuclear respiratory factor-1, cytochrome oxidase B, cytochrome c oxidase-4, uncoupling protein-3, 11β-hydroxysteroid dehydrogenase type-1, glucocorticoid receptor-α, fatty acid synthase, fatty acid binding protein-4, LPL and hormone sensitive lipase (18–24 months post-treatment versus pretreatment levels and controls; P<0.05 to <0.0001).

Conclusions: The decreased expression of genes involved in lipid and mitochondrial metabolism 18–24 months post-ARV treatment in SAT of HIV patients, in conjunction with the increase in uncoupling protein-2 and decrease in cytochrome oxidase B gene expression, provides evidence of mitochondrial dysfunction and a shift to anaerobic metabolism within SAT in EFV-containing ARV regimens.

Introduction

Antiretroviral (ARV) therapies for HIV infection have been associated with abnormal serum lipids, adipocyte gene expression [1–3] and lipodystrophy [4]. The metabolic and adipose tissue (AT) effects observed, however, can vary depending upon the particular ARV regimen used [5]. Tenofovir disoproxil fumarate (TDF)-containing regimens appear to demonstrate less detrimental effects on serum lipids and subcutaneous adipose tissue (SAT) than those containing zidovudine (AZT) [6]. We have previously reported that treatment-naive
HIV-infected subjects randomized to two ARV regimens containing efavirenz (EFV; with AZT plus lamivudine [3TC], or TDF and emtricitabine [FTC]), demonstrate up-regulated expression of many key genes controlling cortisol generation and lipogenesis in SAT 6 months after initiating therapy [7]. Although changes were similar with both treatment regimens, more significant effects were observed following AZT/3TC treatment [7]. The net effect of these changes (to promote lipid storage and limit fatty acid oxidation) was further supported by the observed increase in both peripheral and trunk fat and in the visceral adipose tissue (VAT):SAT ratio [7]. Furthermore, we noted evidence of a shift to anaerobic metabolism with AZT treatment, which showed a significant reduction in cytochrome oxidase B (CYT-B) gene expression, a key mitochondrial gene involved in oxidative phosphorylation (OXPHOS), and increased uncoupling protein (UCP)-2 gene expression, which uncouples electron transport from ATP synthesis [7]. These early changes in SAT are in keeping with reported observations of an increase in both peripheral fat and trunk fat 6 months after therapy [5,8,9], which have been interpreted as evidence for a return to health.

In order to study the accumulating effect of more prolonged exposure to ARV therapies on SAT, we retested the patients 18–24 months after randomization to the two treatment groups. We were particularly interested in determining whether up-regulation of genes involved in adipocyte differentiation and lipid accumulation would persist, thereby explaining reports of higher metabolic syndrome prevalence in ARV-treated HIV patients [10,11]. Such a prospective study at different time points after randomized ARV treatments has not yet been published. We also tested HIV-treatment-naive patients taking an ARV regimen containing abacavir (ABC), 3TC and EFV 18–24 months after initiating treatment as a further comparison group.

Methods

The study obtained ethical approval from the South Birmingham Regional Ethics Committee (Birmingham, UK). Patients (n=32) naive to ARV therapy and eligible for treatment, according to the British HIV Association guidelines, were recruited from the HIV clinic (University Hospitals Birmingham Foundation Trust, UK). All patients gave written consent to participate, with prior explanation from interpreters provided where necessary. Patients were randomized to receive either AZT plus 3TC (as proprietary preparation Comcod) plus EFV twice daily dosage (15 patients) or TDF plus FTC (as proprietary preparation Truvada) plus EFV once daily dosage (17 patients). A further group of 12 patients who had been on their first ARV regimen containing ABC plus 3TC (in proprietary preparation Kivexa) plus EFV were also tested. HIV antibody-negative controls (n=15) were matched for age, ethnicity and gender.

Exclusion criteria included fasting glucose >6.1 mmol/l, weekly alcohol intake >28 units (male) or 21 units (female), intake of lipid lowering drugs, glucocorticoids or any drugs that may affect lipid metabolism (patients were asked to discontinue lipid-lowering drugs for ≥6 weeks), hypothyroidism, creatinine >150 mmol/l and alanine aminotransferase >5× the upper limit of normal, anaemia, >10% loss in body weight in the preceding 6 months, hepatitis B and C and any AIDS-defining disease. A full history was taken from each subject prior to the study, including alcohol intake, concomitant medication and any cardiovascular disease (CVD) and/or type 2 diabetes mellitus (T2DM) history in first degree relatives.

Subjects were tested prior to and 5–7 months post-therapy [7] and again 18–24 months post-therapy. A total of 12 treatment-naive patients who had been started on ABC/3TC/EFV were also tested post-therapy. Demographic details of the patients and controls are given in Table 1.

Routinely, fasted patients were admitted to the Wellcome Trust Research Facility at Queen Elizabeth Hospital (Birmingham, UK) in the morning. Blood pressure, body mass index and waist:hip ratio were measured. Fasting blood samples were taken for a lipid profile, liver function test, thyroid function test, urea and electrolytes, blood glucose, full blood count, CD4+ T-cell count and viral load measurement. Levels of lipoprotein(a), non-esterified fatty acid, glucose, insulin, adiponectin, leptin, urea, creatinine and electrolytes (including chloride and bicarbonate) were also measured. Details of assay methodology have previously been reported [7]. Serum adiponectin, leptin and insulin concentrations were measured by radioimmunoassay using commercially available kits (Millipore Corporate Headquarters, Billerica, MA, USA; intra-assay coefficients of variation 5%, 4.7% and 4%, respectively). Insulin resistance was calculated from fasting insulin and glucose using the homeostasis model assessment-insulin resistance [12].

Subjects underwent whole-body DEXA scanning for body-fat distribution and single slice CT scanning at L4 to measure SAT and VAT. DEXA scan results are expressed as a ratio of peripheral fat (arms plus legs) or trunk fat to lean body mass (LBM). SAT biopsies (n=3) from the iliac crest were performed through a 1 cm incision under local anaesthesia and the samples snap frozen and stored at -80°C until assayed. Total RNA was extracted and reverse transcribed to complementary DNA (cDNA). Gene expression was
quantified using real-time PCR, relative to an internal housekeeping gene (18S), as previously reported [7]. Data were obtained as cycle threshold (ct) values (ct=cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine Δct values (Δct=[ct of the target gene]−[ct of the housekeeping gene]). Data were expressed as arbitrary units using the following transformation (expression =10^[Δct/2−Δct] arbitrary units) or fold change relative to specific control, where fold change=2^[difference in Δct].

At the end of the study, subjects were given a single bolus of 50 IU heparin/kg body weight. Blood was taken in heparinized tubes on ice for estimation of hepatic lipase (HL) and lipoprotein lipase (LPL) prior to and 15 min post-injection. Total lipase and HL activities were measured using a fluorometric assay (Progen Biotechnik GmbH, Heidelberg, Germany). Total lipase and HL activities (expressed as nmol free fatty acids released/ml post-heparin plasma/h) were calculated from a standard curve provided by the kit and a standard of known total LPL and HL activity. The activity in this standard was determined using radioactive triolein in a glycerol-based assay with selective inhibition of LPL activity with a specific monoclonal antibody (kindly donated by JD Brunzell, University of Washington, Seattle, WA, USA). LPL activity was calculated by subtracting HL from total lipase activity. This does not take account of other potential lipases in the serum, and therefore may be more accurately described as ‘non-HL activity’.

Categorical data were compared using the Fisher’s exact test and continuous data with analysis of variance. All statistical analyses on gene expression were performed on Δ values. Inter-group comparisons were made by Mann–Whitney U test, and on related samples by Wilcoxon signed-rank test. Friedman tests were used to analyse the change in the levels of variables over time. Individual Wilcoxon tests were then utilized to highlight any significant differences between pairs of time points. The P-values quoted are not corrected for multiple comparisons; however, the results that remained significant after standard Bonferroni adjustment are indicated in the relevant tables. Because the standardized Bonferroni adjustment is based on an assumption of independence, this is an over-correction for these data that demonstrate substantial correlation. Correlations were by Spearman’s rank test.

Genes profiled
Genes profiled included those involved in adipocyte glucocorticoid and lipid metabolism, adipocyte differentiation and mitochondrial respiratory function.

Genes involved in glucocorticoid generation, essential for adipocyte differentiation
Genes profiled included 11β-hydroxysteroid dehydrogenase type-1 (11βHSD-1), which regenerates active glucocorticoid cortisol from inactive cortisone [13], hexose 6-phosphate dehydrogenase (H6PDH), which generates the essential cofactor NADPH for 11βHSD-1 [14], and glucocorticoid receptor (GR)-α.

Genes involved in adipocyte differentiation and lipid metabolism
Genes profiled included peroxisome proliferator-activated receptor (PPAR)-γ, which induces
pre-adipocyte differentiation and stimulates fatty acid flux, triglyceride storage and insulin sensitivity, fatty acid synthase (FAS), fatty acid binding protein (FABP)-4, LPL, hormone sensitive lipase (HSL), glucose receptor (GLUT)-4, which is the predominant isoform of the glucose transporter mediating insulin-mediated glucose uptake, acetyl coenzyme A carboxylase (ACC)-1, which is the rate limiting enzyme controlling lipogenesis and ACC-2, a key negative regulator of β-oxidation through malonyl coenzyme-A-mediated inhibition of free fatty acid delivery into the mitochondrion.

**Mitochondrially related genes**

Mitochondrially related genes that were profiled included human citrate synthase (HCS), which catalyses acetyl coenzyme A to citrate, a key enzyme in the citric acid (TCA) cycle and a marker of mitochondrial mass [15], PPAR-γ coactivator (PGC)-1α, which is essential for mitochondrial biogenesis [16] and plays a critical role in regulating tissue-specific biological processes co-ordinating it with mitochondrial oxidative metabolism, nuclear respiratory factor-1 (NRF-1), which regulates respiratory gene transcription [17] and also controls mitochondrial transcription factor A (TFAM) [18]. TFAM regulates mitochondrial DNA transcription and is also responsible for the transcription of mitochondrially encoded cytochrome c oxidase (COX) subunits I, II and III [18]. PGC-1α, NRF-1 and TFAM are therefore closely involved with nuclear control of mitochondrial function [18]. Other mitochondrial genes measured included NADH dehydrogenase (ND) subunit 1 (respiratory chain complex I); CYT-B (complex III) and COX-3 (complex IV), both involved in OXPHOS, nuclear encoded COX-4 (complex IV), UCP-1–3, which uncouple electron transport from ATP synthesis and protect against free radical accumulation, and ATP synthase subunit 5s (ATP5s) in complex V of the OXPHOS pathway.

**Results**

Patients randomized to AZT or TDF-containing regimens and controls were matched for age, gender, ethnicity, weight and body mass index, and had similar family histories of CVD or T2DM, as well as pretreatment CD4+ T-cell count, viral loads, peripheral fat/LBM, trunk fat/LBM, VAT or SAT (Table 1). HIV treatment-naïve patients had lower HDL cholesterol (median 1.24 mmol/l [IQR 1.0–1.45] versus controls 1.4 mmol/l [IQR 1.3–1.9]; P=0.02) and plasma LPL activity (median 311 nmol/ml/h [IQR 211–374] versus control 602 nmol/ml/h [IQR 395–652]; P=0.0001), but were similar in all other biochemical and hormonal parameters (data not shown). Patients 18–24 months post-ABC treatment did not differ significantly with regard to anthropometric and biochemical characteristics measured (Table 1).

A total of 9 patients taking AZT and 10 taking TDF were retested 18–24 months post-treatment. Two AZT patients switched treatment prior to retesting, one changing to ABC/3TC plus atazanavir/ritonavir, the other to AZT/TDF plus lopinavir/ritonavir, at the time of testing. These two patients were retested at 18–24 months and incorporated into the analysis, as it was carried out on an intention-to-treat basis. The remaining 13 patients either were lost to follow-up (9 patients), left the country (2 patients) or refused retesting (2 patients). Intergroup analysis of 18–24 month versus baseline or 6 months was carried out on paired data, thus excluding any subjects who did not complete the full treatment course.

**AZT treatment increased total cholesterol** (median 4.2 mmol/l [IQR 3.8–4.9] at baseline and median 4.8 mmol/l [IQR 4.5–6.0] at 18–24 months post-treatment; P=0.01) and HDL cholesterol (1.4 mmol/l [IQR 0.9–1.6] at baseline and 1.6 mmol/l [IQR 1.3–2.2] at 18–24 months post-treatment; P=0.01). TDF treatment increased total cholesterol (median 3.9 mmol/l [IQR 3.3–4.4] at baseline and median 4.8 mmol/l [IQR 4.2–5.2] at 18–24 months post-treatment; P=0.016) and HDL levels (median 1.2 mmol/l [IQR 1.0–1.4] at baseline and median 1.5 mmol/l [IQR 1.2–1.7] at 18–24 months post-treatment; P=0.017). Serum adiponectin and leptin levels significantly decreased following 18–24 months of AZT treatment only (P=0.02; Table 2), with significantly reduced post-treatment LPL levels versus controls in all three treatment groups (AZT, TDF and ABC; Table 2) and post-18–24 month levels for AZT (P=0.008) and TDF (P=0.038) versus baseline (Table 2). Changes in VAT (P=0.002), adiponectin (P=0.04), leptin (P=0.04) and LPL (P=0.008) for AZT treatment and SAT (P=0.007), and LPL (P<0.05) for TDF treatment groups remained significant when analysed over the three time periods of baseline, 6 months and 18–24 months (Tables 2 and 3).

No statistically significant weight changes were observed, with changes in body fat distribution shown in Table 3. There was a significant gain in VAT (P=0.012) and in VAT:SAT ratio (P=0.036) after 18–24 months of AZT versus baseline. There was a significant increase in SAT (P=0.047) and peripheral fat/LBM ratio (P=0.017) 18–24 months post-TDF treatment. No patient had clinical evidence of lipodystrophy. However, two AZT patients lost >25% of their peripheral fat/LBM (29.6% and 34%) and SAT (25% and 35%) 18–24 months post-treatment, whereas there appeared to be no obvious change in weight or gene profile of either patient throughout the study.
Gene expression changes 18–24 months post-AZT and TDF treatment, expressed as fold changes from baseline (≥1), are shown in Figure 1, with expression of each gene at 18–24 months compared to its respective baseline value. As noted in Figure 1, a similar expression pattern of genes controlling cortisol, fatty acid metabolism, glucose entry and OXPHOS was observed with both AZT and TDF treatment groups. Also, for both treatments, there was a significant up-regulation in H6PDH gene expression (P=0.012) and down-regulation of several other genes involved with cortisol, fatty acid and glucose metabolism (Figure 1A). Changes in mitochondrial gene expression relative to baseline levels are shown in Figure 1B. Following 18–24 months of treatment, there was significant up-regulation of PGC-1α (AZT P=0.008 and TDF P=0.005) and ND-1 (TDF P=0.036) and down-regulation of CYT-B (AZT P=0.008 and TDF P=0.009), NRF-1 (TDF P=0.017), COX-4 (AZT P=0.008 and TDF P=0.007), COX-3 (AZT P=0.02) and UCP-3 (AZT P=0.028) versus baseline levels (Figure 1B). The direction of the pattern gene expression for AZT and TDF treatments over time (6 months and 18–24 months compared with baseline) can be observed in Additional file 1.

Figure 2 depicts gene expression changes observed for all three treatment groups (fold change relative to controls). A similar pattern of gene expression in genes controlling cortisol, fatty acid metabolism and glucose entry was observed across all treatment groups.
Gene expression of H6PDH ($P=0.002–0.001$) was increased and 11$\beta$HSD-1, GR-α and FAS decreased in all groups ($P=0.04–0.0001$). In addition, there was significant down-regulation of PPAR-$\gamma$ (AZT $P=0.033$ and TDF $P=0.016$), LPL (AZT $P=0.005$ and TDF $P=0.0001$), HSL and FABP-4 (TDF $P=0.026$) and ACC-2 (ABC $P=0.027$; Figure 2A).

Expression of genes involved in mitochondrial metabolism showed a very similar pattern in all three treatment groups (Figure 2B). All treatment groups exhibited increased expression of PGC-1α ($P=0.0001$), and decreased COX-4 ($P=0.0001$) and UCP-3 (AZT $P=0.003$, TDF $P=0.04$ and ABC $P=0.001$). UCP-2 was up-regulated with AZT ($P=0.007$) and ABC ($P=0.005$). CYT-B was down-regulated following AZT and ABC treatment ($P=0.02$ and $P=0.004$, respectively), whilst TDF treatment led to increased ND-1 ($P=0.001$) and reduced NRF-1 gene expression ($P=0.04$).

Comparison between the three time points (0, 6 and 18–24 months) carried out looking at expression

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**Figure 1.** Changes in genes controlling adiopocyte maturation and mitochondrial transport with AZT- or TDF-containing regimens

(A) Genes controlling adipocyte maturation. (B) Genes controlling mitochondrial electron transport. Results are presented as relative fold changes in gene expression (HIV-infected, treatment-naive = 1 [baseline]). Analysis of 18–24 months versus baseline was carried out on paired data, therefore excluding any subjects who did not complete the full treatment course. Comparisons by Wilcoxon signed-rank tests on related samples, $P<0.05$. ACC, acetyl coenzyme A carboxylase; ATP5s, ATP synthase subunit 5s; AZT, zidovudine; COX, cytochrome c oxidase; CYT-B, cytochrome oxidase B; FABP-4, fatty acid binding protein 4; FAS, fatty acid synthase; GLUT-4, glucose receptor-4; GR-α, glucocorticoid receptor-α; HCS, human citrate synthase; HSL, hormone sensitive lipase; H6PDH, hexose 6-phosphate dehydrogenase; LPL, lipoprotein lipase; ND1, NADH dehydrogenase subunit 1; NRF-1, nuclear respiratory factor-1; PGC-1α, peroxisome proliferator-activated receptor-$\gamma$ coactivator-1α; PPAR-$\gamma$, peroxisome proliferator-activated receptor-$\gamma$; TDF, tenofovir disoproxil fumarate; TFAM, mitochondrial transcription factor-A; UCP, uncoupling protein; 11$\beta$HSD-1, 11$\beta$-hydroxysteroid dehydrogenase type-1.
Figure 2. Changes in genes controlling adipocyte maturation and mitochondrial electron transport with AZT-, TDF- or ABC-containing regimens compared to controls

(A) Genes controlling adipocyte maturation. (B) Genes controlling mitochondrial electron transport. Results are presented as fold changes in gene expression (control = 1). Comparisons by Mann–Whitney U test: *P<0.005; **P<0.0005; ***P<0.05. ABC, abacavir; ACC, acetyl coenzyme A carboxylase; ATP5s, ATP synthase subunit 5s; AZT, zidovudine; COX, cytochrome c oxidase; CYT-B, cytochrome oxidase B; FABP-4, fatty acid binding protein 4; FAS, fatty acid synthase; GLUT-4, glucose receptor-4; GR-α, glucocorticoid receptor-α; HCS, human citrate synthase; HSL, hormone sensitive lipase; H6PDH, hexose 6-phosphate dehydrogenase; LPL, lipoprotein lipase; ND1, NADH dehydrogenase subunit 1; NRF-1, nuclear respiratory factor-1; PGC-1a, peroxisome proliferator-activated receptor-γ coactivator-1α; PPAR-g, peroxisome proliferator-activated receptor-γ; TDF, tenofovir disoproxil fumarate; TFAM, mitochondrial transcription factor-A; UCP, uncoupling protein; 11βHSD-1, 11β-hydroxysteroid dehydrogenase type-1.
of genes involved in lipid and mitochondrial metabolism yielded significant differences for a number of genes, as highlighted in Additional file 2.

Gene expression from the 18–24 month AZT and TDF groups were further compared and contrasted against uninfected controls, in addition to baseline gene expression levels (Table 4). There was a significant increase in expression of all genes controlling adipocyte maturation investigated 6 months post-ARV treatment compared with pretreatment levels (Table 4). In addition, PGC-1α, TFAM, ND-4 and COX-4 were up-regulated while CYT-B was down-regulated at 6 months compared to pretreatment levels. However, at 18–24 months there was a significant up-regulation of PGC-1α, UC-P-2 and H6PDH, and down-regulation of NRF-1, CYT-B, COX-4, UCP-3, 11βHSD-1, GR-α, FAS, FABP-4, LPL and HSL, observed, compared with both pretreatment levels and healthy controls (Table 4). Changes in PGC-1α (P<0.001), NRF-1 (P=0.002), CYT-B (P<0.001), COX-4 (P<0.001), UCP-3 (P=0.002), PPAR-γ (P=0.002), H6PDH (P=0.002), 11βHSD-1, GR-α, FABP-4, LPL, HSL (P<0.001) and ACC-2 (P=0.012) remained significant when analysed over the three time points of baseline (0 months), 6 months and 18–24 months (Table 4).

When all three treatment groups (AZT, TDF and ABC) were combined and examined solely across the 18–24 month time-point, a significant up-regulation of PGC-1α (P=0.0001), ND-1 (P=0.05), UCP-2 (P=0.002), and H6PDH (P=0.0001), and a significant down-regulation of NRF-1 (P=0.003), CYT-B (P=0.007), COX-4 (P=0.0001), UCP-3 (P=0.001), 11βHSD-1, GR-α, FAS, (all P<0.0001), FABP-4 (P=0.02), LPL (P=0.001), HSL (P=0.02) and ACC-2 (P=0.024) gene expression was observed versus uninfected controls (data not shown).

Intergroup comparison at 18–24 month post-treatment yielded significant differences in gene expression of adipocyte genes involved with mitochondrial respiratory chain, glucocorticoid generation and fatty acid metabolism

Table 4. Expression of adipocyte genes involved with mitochondrial respiratory chain, glucocorticoid generation and fatty acid metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>HIV, baseline</th>
<th>P-value, baseline HIV versus control</th>
<th>HIV, 6 months</th>
<th>P-value, HIV versus baseline</th>
<th>HIV, 18–24 months</th>
<th>P-value, HIV versus baseline</th>
<th>P-value, all three time points</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC-1α</td>
<td>10 (6–13)</td>
<td>10 (8–16)</td>
<td>0.019</td>
<td>40 (31–57)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>NRF-1</td>
<td>74 (62–84)</td>
<td>93 (69–120)</td>
<td>0.003</td>
<td>48 (37–74)</td>
<td>0.021</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFAM</td>
<td>0.11 (0.10–0.15)</td>
<td>0.14 (0.11–0.19)</td>
<td>0.012</td>
<td>0.16 (0.11–0.26)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ND-1</td>
<td>391 (333–427)</td>
<td>430 (394–584)</td>
<td>0.045</td>
<td>508 (465–876)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>ND-4</td>
<td>56 (37–86)</td>
<td>44 (26–107)</td>
<td>0.002</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>CYT-B</td>
<td>43.6 (28.9–60.1)</td>
<td>62.5 (51.6–83.0)</td>
<td>0.004</td>
<td>25.6 (15.7–39.6)</td>
<td>&lt;0.0011</td>
<td>0.017</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>COX-3</td>
<td>114 (79–139)</td>
<td>148 (89–253)</td>
<td>0.003</td>
<td>89 (50–157)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX-4</td>
<td>5.2 (2.2–6.6)</td>
<td>4.2 (2.1–9.3)</td>
<td>0.001</td>
<td>1.4 (1.1–2.2)</td>
<td>&lt;0.0011</td>
<td>0.003</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>UCP-2</td>
<td>1.1 (0.7–1.3)</td>
<td>1.2 (0.8–1.5)</td>
<td>0.005</td>
<td>1.1 (0.7–2.0)</td>
<td>0.003</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCP-3</td>
<td>6.0 (2.0–8.1)</td>
<td>3.5 (2.0–6.9)</td>
<td>0.005</td>
<td>3.2 (2.1–5.8)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>ATP5a</td>
<td>1.1 (0.61–1.5)</td>
<td>0.82 (0.57–1.1)</td>
<td>0.005</td>
<td>0.08 (0.6–1.0)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCS</td>
<td>0.77 (0.62–1.07)</td>
<td>1.22 (0.73–1.65)</td>
<td>0.005</td>
<td>0.8 (0.6–1.0)</td>
<td>0.017</td>
<td>0.003</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>16.7 (10.8–24.8)</td>
<td>19.7 (12.4–25.8)</td>
<td>&lt;0.0011</td>
<td>9.6 (4.3–13.7)</td>
<td>0.005</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6PDH</td>
<td>1.06 (0.64–1.95)</td>
<td>1.12 (0.57–1.37)</td>
<td>&lt;0.0011</td>
<td>3.2 (2.1–5.8)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>11βHSD1-1</td>
<td>0.57 (0.29–0.74)</td>
<td>0.28 (0.12–0.37)</td>
<td>0.001</td>
<td>0.08 (0.04–1.19)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>GR-α</td>
<td>10.4 (7.7–16.4)</td>
<td>10.8 (6.6–14.3)</td>
<td>&lt;0.0011</td>
<td>3.2 (2.6–4.8)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
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<tr>
<td>FAS</td>
<td>48.7 (32.4–105.0)</td>
<td>88.0 (44.8–193)</td>
<td>&lt;0.0011</td>
<td>5.2 (2.8–10.4)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
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<tr>
<td>FABP-4</td>
<td>447 (232–706)</td>
<td>475 (360–673)</td>
<td>0.005</td>
<td>303 (181–335)</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td>&lt;0.0011</td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>129 (111–221)</td>
<td>196 (122–237)</td>
<td>0.005</td>
<td>49 (25–97)</td>
<td>0.012</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSL</td>
<td>0.30 (0.18–0.51)</td>
<td>0.28 (0.18–0.60)</td>
<td>0.001</td>
<td>0.14 (0.09–0.29)</td>
<td>0.012</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLUT-4</td>
<td>2.0 (1.2–3.4)</td>
<td>2.6 (1.2–4.5)</td>
<td>0.001</td>
<td>1.7 (0.8–2.7)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC-1</td>
<td>0.59 (0.44–2.87)</td>
<td>0.93 (0.49–1.56)</td>
<td>0.023</td>
<td>0.5 (0.3–0.6)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACC-2</td>
<td>13.0 (10.5–28.1)</td>
<td>14.0 (9.4–21.1)</td>
<td>0.001</td>
<td>10.8 (6.3–19.4)</td>
<td>0.012</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
expression of CYT-B between AZT and TDF (P<0.05) and GR-α between ABC and both AZT and TDF (P=0.001 and P=0.003, respectively), in addition to revealing correlations between gene expression of PGC-1α and NRF-1 (rho 0.74, P=0.0001), TFAM (rho 0.69, P=0.0001), COX-3 (rho 0.48, P=0.007), COX-4 (rho 0.55, P<0.001) and UCP-3 (rho 0.57, P=0.001) 18–24 months post-treatment. UCP-2 gene expression correlated with NRF-1 (rho 0.6, P=0.001), TFAM (rho 0.68, P=0.0001), COX-4 (rho 0.63, P=0.0001) and ATP5s (rho 0.63, P=0.0001). Strong correlations (P<0.001) were also observed between many of the genes controlling cortisol, fatty acid metabolism and GLUT-4 within the 18–24 month time-point (data not shown).

Discussion

The present study reports reduced expression of genes involved in lipogenesis, fatty acid influx/efflux and glucose entry in SAT of HIV patients 18–24 months post-ARV treatment with regimens containing two nucleoside analogues and EFV. Down-regulation of these genes was observed 18–24 months post-treatment versus pretreatment (baseline) levels and controls, regardless of the nucleoside backbone of the drug administered. These changes were paralleled by a significant rise in VAT in the AZT-containing group or SAT in the TDF-containing regimen. As we do not have pretreatment body fat measurements, body fat changes in the ABC-containing regimen could not be accurately assessed.

We also observed evidence of mitochondrial respiratory chain dysfunction, following ARV therapy, with more genes being significantly affected following AZT treatment compared with TDF. There was significant down-regulation of mitochondrial encoded CYT-B (complex III) by AZT and ABC treatment, and nuclear encoded COX-4 (complex IV) in all the three treatment groups versus controls, suggesting a nuclear compensatory mechanism may be at work, as mitochondrial oxidative capacity alone cannot account for the observed reduction in nuclear encoded gene expression. The increase in UCP-2 18–24 months post-AZT and ABC treatment versus controls, suggests some impairment in the ability to synthesize ATP. That the rise in UCP-2 did not reach significance in the TDF group may reflect the small number of subjects studied. However, this is in keeping with previous studies reporting that TDF-containing regimens have less association with lipodystrophy, a condition believed to be strongly linked with mitochondrial dysfunction [19]. The strong correlation observed between gene expression of UCP-2, NRF-1, TFAM (critical for respiratory gene transcription and mitochondrial abundance [18]) and ATP5s, suggests that up-regulation of UCP-2 in this study is related to mitochondrial respiratory chain activity [20] and provides evidence of disturbance in mitochondrial OXPHOS with all three treatments. The significant decrease in adiponectin and leptin levels post-AZT treatment may provide further evidence for a disruption in OXPHOS and increased reactive oxygen species (ROS) production [21], though the effect on adiponectin and leptin release may not be wholly mediated by ROS [22]. In contrast to Pace et al. [23], who found elevated UCP-1 mRNA expression in SAT of HIV patients on ARV-containing protease inhibitors, UCP-1 was not expressed in any of our treatment regimens.

The increased PGC-1α gene expression observed across all three treatment groups reflects the important role of this gene in mitochondrial biogenesis and the regulation of tissue energy requirement and production [16,24,25]. The strong positive correlation of PGC-1α gene expression observed with NRF-1, TFAM and COX-4 within the 18–24 month time-point (combining all three treatment groups [AZT, TDF and ABC]) re-emphasizes the known role of PGC-1α in the regulation of these genes [18]. Thus, this observation may be interpreted as a physiological response by SAT to address an imbalance between energy production and requirement.

Disturbance in expression of mitochondrial genes involved in the AZT group was evident 6 months post-therapy [7]. There was a significant reduction in CYT-B (complex III) gene expression and a compensatory up-regulation of nuclear-encoded COX-4 (complex IV) gene expression, confirming previous reports that changes in mitochondrial function can be demonstrated early in the course of ARV therapy with some regimens [2]. Patients taking TDF did not show such changes. However, 18–24 months post-ARV therapy, changes in mitochondrial gene expression in the two groups were broadly similar and comparable to those noted in patients taking ABC for a similar length of time, reflecting the prevalence of body fat disturbances when these nucleosides are used in ARV regimens [5,26–30]. Thus the differences observed in the present study between treatment regimens containing either AZT, TDF or ABC, with respect to gene expression and body fat distribution, and also between AZT and TDF with respect to adiponectin and leptin levels, highlight the important role of nucleoside/nucleotide-containing ARV regimens on lipid and mitochondrial metabolism in vivo.

The fat content of adipocytes can be considered a balance between supply (fatty acid influx and synthesis) and the demand for fat utilization (fatty acid β-oxidation or efflux). We have previously shown that 6 months...
post-ARV treatment, there is an up-regulation of all genes controlling fatty acid influx and lipogenesis and a significant increase in serum LPL activity [7], which may explain the gain in both peripheral and trunk fat reported early on in several studies of ARV therapy [5,8,9]. Our observations at 18–24 months are more complex and show a reduction in lipid synthesis (ACC and FAS) and free fatty acid availability from circulating triglyceride hydrolysis (LPL gene expression and post-heparin LPL activity). This was, however, combined with decreased lipolysis (HSL), perhaps being driven by decreased cortisol generation and action (11βHSD-1 and GR-α) and impaired oxidative capacity with mitochondrial chain dysfunction. A disruption in the mitochondrial OXPHOS system, as suggested by the increased UCP-2, would also be expected to inhibit lipolysis and reduce fatty acid β-oxidation [31]. It is plausible that the net effect of these observations is a shift towards a more pronounced limitation of triglyceride mobilization and utilization, which, if greater than the decreased synthesis, may explain the continued increase in fat mass we observed. It is a possibility that lipolysis and re-esterification increase in concert, contributing to a futile cycle thus limiting any net change in triglyceride accumulation. We did not observe any significant changes in GLUT-4 expression, which might have enhanced intracellular glucose availability that may have driven this process. However, dynamic studies of glucose transport were not performed in this study. There was a median gain in SAT in both AZT and TDF, but only significant in the latter. There was, however, a ≥20% reduction in SAT in two of nine patients receiving AZT without clinical lipoatrophy, which may be an early sign of subsequent clinical lipoatrophy, and does not occur in all patients taking AZT-containing regimens [32]. We speculate that an imbalance between supply and demand of fatty acids may partly explain the observed changes in SAT at 18–24 months post-ARV therapy.

Gene expression may not accurately reflect changes in protein expression because of the effects of post-transcriptional regulation or post-translational modifications of factors regulating the expression of mitochondrial OXPHOS proteins and also in the nuclear-mitochondrial response to cellular events [18]. Furthermore, we cannot extrapolate findings from SAT to VAT, as different regional fat depot varies in their mitochondrial content [31] and metabolic responses [33,34]. However, it is possible that the same mechanisms may also be operating within this system [35]. Moreover, changes in gene expression in AT as a whole, may not necessarily reflect changes within specific cells. Thus the mitochondrial content of tissue macrophages and other inflammatory cells, whose numbers are increased after ARV treatment [36], are different from those found in white adipocytes. There are also changes in mitochondrial number and mitochondrial biogenesis during adipocyte maturation [31]. However, while we cannot extrapolate our findings to subcutaneous adipocytes, our study may still provide insights on the effect of ARV on SAT as a whole.

Evidence for mitochondrial dysfunction [24] and depletion [23,37,38] have mainly implicated the nucleoside component of the ARV regimens. Moreover, most studies on gene expression have been cross-sectional, have included patients with evidence of lipodystrophy and report on regimens containing protease inhibitors. Giralt et al. [1] reporting on non-lipodystrophic patients taking ARV regimens that contained either protease inhibitors or non-nucleoside reverse transcriptase inhibitors for a median 37 months, found up-regulation of PGC-1α and UCP-2 and down-regulation of COX-2, COX-4, LPL and GLUT-4, compared to HIV-negative controls, which are results similar to our study. Kratz et al. [8] in a prospective study of patients without lipoatrophy, showed an increase in 11βHSD-1 concentration in both thigh and abdominal AT 12 months post-ARV treatment, in agreement with our findings.

In accordance with the observations made in the present study, current literature has also reported PPAR-γ to be down-regulated compared with HIV-negative controls [1,39], yet remaining unchanged [8,23] or elevated [1] in HAART-treated HIV patients compared with treatment-naïve HIV patients. None of these studies, however, have specifically examined the role of non-nucleoside containing ARV regimens in adipocyte gene expression.

Evidence suggests that EFV-containing regimens can impair mitochondrial respiration in vitro [40] and cause a greater degree of lipoatrophy, regardless of the nucleoside backbone [30,41,42], when compared against protease inhibitor containing regimens, suggesting the potential for an additional role for EFV in lipid and mitochondrial metabolism. Furthermore, EFV has also been associated with osteopenia [43]. Even slight impairment of mitochondrial function can increase electron leakage and production of ROS in vitro [44–48]. Should anaerobic metabolism in SAT (observed with all three EFV-containing regimens, albeit to different degrees) persist over several years, it may expose the AT to excessive ROS levels. Although we have not directly assessed ROS production in the present study, the observed changes in gene expression, particularly with the increase in UCP-2 expression, suggest that 18–24 months post-ARV, SAT may be exposed to increased ROS production. Oxidative stress after various ARV regimens has been shown in vitro [22] and may contribute to the emergence of tissue senescence in HIV patients [49]. Chronic ROS exposure may facilitate the emergence of ageing-related diseases, such as cancer and CVD [50,51]. There is
increasing evidence that HIV patients are prone to ageing-related diseases, such as CVD and T2DM [52–54] at a younger age, and in lower risk HIV populations, such as women [55–57]. The present study also allows speculation that a possible increase in ROS with EFV-containing ARV regimens may contribute to the increased incidence of ageing-related disorders in younger HIV populations, potentially contributing to the increasing reports of non-AIDS defining cancers in HIV-infected patients [54]. As previously highlighted, the metabolic changes observed in the current literature between EFV-containing regimens and those containing, for example, a protease inhibitor, suggest there may be additional consequences of EFV treatment. Whether these changes result from EFV-containing regimens specifically, or are common to other or all ARV regimens, requires further study.

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Disclosure statement

The authors declare no competing interests.

Additional files

Additional file 1: A supplementary figure illustrating the direction of the pattern of gene expression for AZT and TDF treatments over time compared with baseline can be found at http://www.intmedpress.com/uploads/documents/AVT-11-OA-2222_McGee_Add_file1.pdf


References


