Lipodystrophy (LD) is a well-defined syndrome that affects many HIV patients treated with highly active antiretroviral therapy (HAART).1–4 This syndrome is a progressive process that appears about 6 months after HAART is begun and becomes clinically significant after 1 year of therapy.5 It remains controversial whether LD is more common in patients taking protease inhibitor (PI)1,6–8 or thymidine analogue9–11 containing therapy. However, as antiretroviral therapy is usually given in combination, the relative contribution of PI and thymidine analogues in causing LD is unclear.9,12

Reversibility of LD has been evaluated in randomized trials and cohorts using various methods involving both PI switching7,9,13 and thymidine analogue switching.11,14,15 These studies were conducted...
over a relatively short time period and resulted in beneficial alterations of metabolic parameters, but only small changes in LD-related body composition. Changes in body shape associated with HAART therapy in a cohort of 277 patients who were followed for 2 years indicated that the rate of progression of LD was greater in those patients treated with stavudine (d4T) than those with zidovudine (ZDV). However, PI therapy in addition to nucleoside reverse transcriptase inhibitor (NRTI) treatment accelerated this rate of progression.

There remains a need for further long-term investigations into possible alterations in antiretroviral-associated LD. The PIILR long-term follow-up study involved a well-described cohort of patients initially enrolled in a randomized controlled trial of stopping or continuing PI-based therapy. During the 24-week randomized phase of the PIILR trial, abdominal fat decreased but so did limb fat mass. Longer follow-up was undertaken to determine if overall improvement in LD could be achieved and to verify which factors influenced changes in body composition.

METHOD

Study Population

This study involved the long-term follow-up of patients with clinically assessed LD while on suppressive HAART, who were enrolled in a randomized, multicenter study comparing the effect of PI substitution with continued PI-based treatment. In the original PIILR study, all patients continued current nucleoside analogue therapy. Sixty percent of patients were randomized to switch PI therapy and 40% to continue. Switch group patients replaced PI(s) with open-label abacavir (300 mg twice daily), nevirapine (200 mg daily for 2 weeks, then 200 mg twice daily), adefovir (60 mg daily, with L-carnitine supplementation 500 mg daily) at baseline and with hydroxyurea (500 mg twice daily) from week 4, while continuing their existing nucleoside analogues. This combination was selected on the basis of concerns regarding the maintenance of adequate viral suppression in extensively pretreated patients who were discontinuing effective PI therapy. In the original PIILR trial, 71% of patients randomized to continue PI therapy elected to switch to a non-protease regimen at week 24, making this a cohort of predominantly PI-sparing patients with clinically evident LD. After week 24, changes to antiretroviral therapy were at the discretion of the clinician and patient. During the follow-up phase, patients attended for metabolic and body scanning. Due to the logistics of arranging continued body scanning at some sites, not all patients had imaging data available. This analysis comprises those participants who completed both the body composition and metabolic measurements to week 120.

Assessments

Details of all prior antiretroviral therapy were recorded at baseline. Weight, safety bloods (complete blood count, electrolytes, liver enzymes, urea, creatinine, creatine kinase, phosphate, amylase), and adverse events were assessed every 3 months. CD4 T-lymphocyte counts, fasting total and high density lipoprotein (HDL) cholesterol, triglyceride, glucose, insulin, and C-peptide levels were determined as previously described every 3 months. Real-time plasma HIV RNA monitoring was performed at each visit using either the Roche Amplicor Monitor assay version 1.0 (Roche Diagnostics, Branchburg, New Jersey, USA; lower limit of detection 400 copies/mL plasma) or Chiron bDNA assay version 3 (Chiron Corporation, Emeryville, California, USA; lower limit of detection 50 copies/mL).

Total and regional body composition was quantified at screening and at weeks 24, 48, and 120 by DEXA (Lunar DPXL; Madison, Wisconsin, USA). Intraabdominal and extraabdominal fat areas at the L4 vertebral level were measured at each study site by single-cut CT. The validated lipodystrophy case definition scoring (LCDS) system is based on demographic, body composition, and metabolic data. The LCDS was calculated at the completion of the study using the available study data. A score of 0 indicates presence of LD, with higher scores reflecting more severe LD.

Analysis

The main objective of the long-term follow-up study was to assess which factors affect long-term changes in different measures of LD. The primary study outcomes were:

- change in limb fat from baseline at week 120 as assessed by DEXA scan, and
change in visceral adipose tissue (VAT) from baseline at week 120 as assessed by CT scan.

The secondary outcomes included changes in lean body mass (LBM), cholesterol (total and HDL), triglycerides, CD4 count, and proportion with detectable viral load from baseline at week 120. In addition, the change in the LCDS from baseline to week 120 was assessed as an alternate measure of LD in a post hoc analysis, as the scoring system had not been developed when the present study’s analysis plan was designed.

Analyses were limited to those patients with DEXA and CT scans at week 120, because these measures were required for the primary objective of the study. Limb fat mass, VAT, and the LCDS were used as indicators of LD severity. Comparisons between parameters were performed using analysis of variance (ANOVA) and t tests. Formal statistical comparisons between the cohorts included and excluded from analyses were not performed because statistically significant differences would not necessarily imply clinically important differences and vice versa. This approach is generally now accepted for comparisons of treatment groups in terms of baseline characteristics in a randomized trial, which are essentially analogous.

Univariate and multivariate linear regressions were used to determine predictors of change in limb fat from baseline to week 120. Initial treatment assignment, triglycerides, and duration on different antiretrovirals were assessed as predictors. The final predictive model was determined using forward stepwise regression. Variables with a univariate p value less than .1 were assessed in multivariate analysis.

RESULTS

Participants

Eighty-one patients were screened and randomized into the PIILR trial. One patient randomized to the continue group was lost to follow-up after baseline, and three patients were lost to follow-up after the week 48 visit. Seventy-seven patients were followed up for 120 weeks. Of these, 45 (28 switch, 17 continue) attended for both metabolic and body composition assessments, whereas 32 only attended for metabolic assessments. This analysis was based on only those patients with both datasets available (Figure 1). Of the 28 patients initially randomized to switch their PI therapy, only 2 reinitiated PI therapy (one at week 77 and one at week 82). Of those 17 patients initially randomized to continue their PI therapy, 16 elected to cease PI therapy after their week 24 visit and only 1 subsequently restarted at week 102. Therefore, only 4 patients were taking PI therapy beyond the week 24 visit in this 120-week follow-up, thereby making this cohort of 45 patients predominantly PI sparing (Figure 1).

Compared to the patients who did not complete body composition analyses at the week 120 visit (Table 1), the baseline characteristics of the studied cohort (n = 45) showed that these patients were all male, had a higher percentage with category C disease, had a slightly longer duration of diagnosed HIV infection, were considerably heavier, and had a slightly greater VAT.

Antiretroviral Therapy

The duration of antiretroviral treatment is summarized in Table 2. All patients were naïve to the antiretroviral drugs abacavir, nevirapine, hydroxyurea, and adefovir prior to enrollment in the trial. The median study duration of adefovir and hydroxyurea was 29 and 32 weeks, respectively, due to the toxicities associated with these antiretroviral agents. Prior study exposure to ZDV was greatest (153 ± 21 weeks), followed by PIs (113 ± 5.4 weeks), and then d4T (86 ± 6 weeks). While on study, more than half of the patients were not taking ZDV and 60% were randomized to stop PI, therefore the mean duration of treatment while on study was greater for d4T.

Measures of LD

Limb fat mass and visceral adipose tissue did not change significantly over the 120-week follow-up, however a significant increase in the LCDS suggested worsening LD over the 120 weeks (p < .001, week 120 vs. baseline; Figure 2). The LCDS is composed of body composition and metabolic parameters; even though independently no factor changed significantly over time, the subtle changes within each parameter may have combined to create a small but statistically significant change.

Lean body mass was analyzed to ensure that fat changes were related to LD, not general weight changes. The lean body mass and weight did not change over the 120 weeks.
Table 1. Baseline characteristics of included and excluded patients in the long-term follow-up (mean ± SEM or %)

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>PIILR cohort not completing body composition long-term follow-up (n = 32)</th>
<th>PIILR cohort with long-term follow-up body composition (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48 ± 2</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>Male (%)</td>
<td>94</td>
<td>100</td>
</tr>
<tr>
<td>Category C disease (%)</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>RNA undetectable (%)</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>HIV duration (years)</td>
<td>7.1 ± 0.7</td>
<td>8.7 ± 0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 ± 2</td>
<td>86 ± 1</td>
</tr>
<tr>
<td>Limb fat mass (kg)</td>
<td>3.9 ± 0.8</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>126 ± 13</td>
<td>136 ± 12</td>
</tr>
</tbody>
</table>
Laboratory Parameters

Control of viral replication was maintained and the lipid and glycemic parameters were not significantly different over the 2.5-year follow-up (Table 3).

Linear Regression Analysis

Linear regression analysis showed no association between the LD indicators and weight, triglycerides, initial randomization group, or ZDV, adefovir, or hydroxyurea usage. However, ongoing d4T usage over the 120-week follow-up was independently and significantly correlated with greater declines in limb fat mass (-0.46 kg, \( p = .003 \)) and a greater increase in LCDS (3.19, \( p = .021 \)), such that for each year of d4T use, a decrease of 0.46 kg limb fat mass was seen. Longer exposure to PIs prior to trial entry was associated with a decrease in limb fat over the 120 weeks (-0.44 kg, \( p = .045 \)), however longer usage of PIs on study was associated with an increase in limb fat over the 120 weeks (0.82 kg, \( p = .007 \)). Given the short duration of PI use overall on the study (mean of 18 weeks within the 2.5-year follow-up), a few patients with greater recovery of limb fat who were reluctant to stop PIs at week 24 may have biased this outcome.

DISCUSSION

An increasing body of evidence strongly suggests that the use of thymidine analogues and/or PIs significantly contributes to the development of the LD syndrome.1,6,8,11,13–15 More recent studies

Table 2. HIV treatment duration prior to trial entry and total duration at the end of the follow-up (mean ± SEM)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Duration of usage at baseline, weeks ((n = 45))</th>
<th>On-study duration of usage, weeks ((n = 45))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease inhibitors</td>
<td>113 ± 5.4</td>
<td>18 ± 4.0</td>
</tr>
<tr>
<td>Stavudine (d4T)</td>
<td>86 ± 6.1</td>
<td>70 ± 7.9</td>
</tr>
<tr>
<td>Zidovudine (ZDV)</td>
<td>153 ± 21.4</td>
<td>17 ± 6.1</td>
</tr>
<tr>
<td>Abacavir</td>
<td>NA</td>
<td>104 ± 4.4</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>NA</td>
<td>108 ± 4.6</td>
</tr>
<tr>
<td>Adefovir</td>
<td>NA</td>
<td>31 ± 2.5</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>NA</td>
<td>45 ± 5.5</td>
</tr>
</tbody>
</table>

Table 3. Laboratory parameters at baseline and week 120 (mean ± SEM)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline ((n = 45))</th>
<th>Week 120 ((n = 45))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 T-lymphocyte count (×10^9/L)</td>
<td>498 ± 42</td>
<td>490 ± 32</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.6 ± 0.2</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.95 ± 0.03</td>
<td>1.06 ± 0.05</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.60 ± 0.17</td>
<td>3.17 ± 0.15</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>12.29 ± 1.41</td>
<td>12.53 ± 1.02</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.33 ± 0.13</td>
<td>5.24 ± 1.48</td>
</tr>
<tr>
<td>C-peptide (µg/L)</td>
<td>2.96 ± 0.21</td>
<td>3.67 ± 0.27</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>4.3 ± 0.5</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.73 ± 0.08</td>
<td>1.61 ± 0.07</td>
</tr>
<tr>
<td>HIV RNA undetectable (%)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
have suggested reversibility of limb fat may occur with cessation of thymidine analogues.18–20

LD did not improve over the 120-week follow-up in this cohort of treated patients. When LD severity was compared with various factors using linear regression, patients with greater exposure to d4T had reduced limb fat and a higher LCDS over the 120 weeks. The mechanism for this effect has been speculated to be due to nucleoside analogue–induced mitochondrial damage.21,22 In the multivariate model, PI duration prior to study entry was associated with less limb fat, but PI duration dur-

Figure 2. Body composition and lipodystrophy case definition score from baseline to 120 weeks in long-term follow-up patients (mean ± SEM).
ing the 120-week follow-up was associated with greater limb fat over the 120-week period. These conflicting findings may be a reflection of the open-label nature of the study that was conducted at a time when PIs were thought to cause LD, that is, those patients with less severe LD chose to remain on their PIs, whereas those patients with more severe LD chose to discontinue their PIs. Patients were off PIs for the majority of the observation period, so this association should be interpreted cautiously.

LD was not reversed in this long-term strategy of switching from PI-based HAART to predominantly PI-sparing HAART. The apparent loss of limb fat and VAT up to week 48 may have been related to the initial use of adefovir and hydroxyurea and may represent the combined adverse effects of these drugs on LD. Hydroxyurea is known to increase the toxicities associated with nucleoside analogues, and adefovir is associated with weight loss. As most patients discontinued adefovir and hydroxyurea within the first year (due to toxicity), some recovery from these effects may have occurred. Another possible explanation of the loss of visceral adipose tissue seen at week 24 and 48 may be due to the impact of removing PI therapy at week 0 for the switch arm and week 24 for the continue arm, this is explained in greater detail in the original PIILR paper. However, there are potential limitations with the analysis of this cohort in that only 58% of original trial participants completed the body composition analysis at 120 weeks. Also, all patients were drug experienced, with moderate to severe LD at trial entry, a group with the worst prognosis for LD recovery. However, our study used objective measures for changes in LD, compared with many studies that have utilized only subjective assessments of LD.

The finding of a significant increase in the LDCS over the 120 weeks, without significant changes in any other primary or secondary endpoint, may be due to subtle changes that occurred within the LDCS calculation. The factors that comprise the LDCS are age, sex, duration of HIV infection, CDC classification, HDL cholesterol, anion gap, waist:hip ratio, VAT:SAT ratio, trunk:limb ratio, and leg fat percentage. Of these parameters, the sex, age, duration of HIV duration, and CDC classification do not significantly vary over time. However, 4 out of the 6 metabolic and body fat parameters increased over time in the cohort, those being waist:hip ratio, trunk:limb ratio, HDL cholesterol, and anion gap.

The current backbone of antiretroviral therapy utilizes thymidine analogues. The withdrawal of thymidine analogues from patient’s antiretroviral treatment has been examined in the MITOX study. This 24-week study examined the effect of switching d4T or ZDV to abacavir on HIV lipodystrophy. There were significant increases in objectively measured limb fat, but these changes were not apparent subjectively within this 6-month period. The long-term follow-up of the MITOX study reported that over 104 weeks the patients who switched to abacavir at baseline increased limb fat by 35%, whereas those who were randomized to remain on thymidine analogue therapy for the first 24 weeks only improved by 13%. The gain in limb fat in the abacavir group after 2 years of follow-up was also subjectively apparent ($p = .003$).

Long-term exposure to PI therapy prior to trial entry may be a surrogate marker of long-term antiretroviral therapy. Exposure to nucleoside analogues other than d4T or ZDV prior to trial entry was not analyzed as a covariate, but this may explain the multivariate correlation reported between reduced limb fat recovery and longer prior PI exposure. In addition, patients who elected to remain on PI therapy while on study appear to be those patients who had less severe LD and therefore saw no need to change treatment regimens.

It remains unclear whether LD is a single pathogenic process, involving nucleoside analogue-induced mitochondrial toxicity, PI-induced alterations in lipid metabolism and adipocyte apoptosis, or a mixed condition. Similarly, the role of nonnucleosides in this process is unclear, however, numerous cross-sectional and prospective studies have failed to implicate this group of drugs. It is also unclear whether there is an irreversible component to LD. Apart from the MITOX extension study, reversibility of changes of LD has been limited or not apparent, particularly in patients remaining on antiretroviral therapy, in a variety of switch studies. Where reversibility in body fat has been described, the most compelling data come from thymidine analogue withdrawal studies. To date, protease withdrawal strategies have been associated with improvements in lipid or glycemic parameters, but not body fat. Interventions beyond switching antiretroviral agents also need to be examined for
those patients who have limited treatment options due to resistance to various drug classes.

CONCLUSION

In summary, features of LD did not improve over 120 weeks after switching from a PI to a non-PI strategy had been used. However exposure to d4T may adversely influence the syndrome.

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