The aim of this study was to evaluate the influence of three variables – protease inhibitors, stavudine, and the length of combined therapy – on body habitus changes, metabolic effects and bone mineral density in HIV patients treated with highly active antiretroviral therapy (HAART). The onset of possible cardiovascular involvement was considered.

Forty HIV patients (29 men and 11 women, mean age 39.13 ± 7.82 years, range 28-61 years) treated with HAART for 12-43 months were evaluated for fat, lean, bone tissues, immunohematological and cardiovascular alterations.

The differences in fat/lean tissues and bone mineral density were evaluated at dual-energy X-ray absorptiometry (DEXA). Serum lipids and the CD4/CD8 T-cell counts were recorded. ECGs were taken every 6 months; color Doppler echocardiography and color Doppler ultrasounds of the carotid vessels were performed in close chronological sequence with the second DEXA. Statistical analyses included: Student’s t-test, Wilcoxon test, and single-multiple regression analysis.

Thirteen patients presented with fat loss, 7 fat accumulation, and 20 a combined form of both. The changes in the single body districts showed that the decrease in the limb fat is to be attributed to protease inhibitors, while none of the three variables was responsible for the decrease in the upper limb fat. The trunk weight increase was not significant. The decrease in the lean mass of the upper limbs is to be attributed to protease inhibitors, while none of the three variables was responsible for the increase in the lean mass of the upper and lower limbs. The decrease in bone mineral density was not significant. No treatment-related cardiovascular lesions were observed.

In HIV patients treated with HAART for 12-43 months, the decrease in lower limb fat was due to protease inhibitors. Neither osteopenia nor cardiovascular diseases were observed during follow-up.

Key words: Cardiovascular risk; Highly active antiretroviral therapy; Lipodystrophy; Osteopenia.
same period, the patients’ biochemical parameters were also evaluated, together with their CD4/CD8 T-cell counts. The onset of possible cardiovascular diseases was also monitored.

Two years after the last DEXA examination, 38/40 patients were again evaluated for any changes in body habitus.

**Methods**

From November 1996 until June 2000, 40 patients (29 men and 11 women, mean age 39.13 ± 7.82 years, range 28-61 years) – 25 of whom were intravenous drug users and 6 homosexual – were enrolled by our Service in the Department of Infectious Diseases of the University of Rome “La Sapienza” while receiving routine care for their documented HIV infection. All of them were clinically stable, without any concomitant active AIDS-related disorders or opportunistic infections.

Anabolic/steroid drugs were not administered and fasting blood samples were collected from each patient for the CD4/CD8 T-cell count, total cholesterol, HDL cholesterol, triglycerides, amylase and hemoglobin. The total fat, the lean body mass (LBM) of the limbs and the trunk, and the bone mineral density were assessed by means of two DEXA analyses of the body composition and expressed in grams.

Measurements were performed by means of total body scanners (QDR-1000 and QDR-2000, Hologic Inc., Waltham, MA, USA); with this technique it is possible to exactly quantify the tissues in peripheral regions. However, since the abdominal subcutaneous and visceral fat compartments may change in opposite directions in the same patient, a single computed tomography cut at L4 is preferable to DEXA examinations18.

All the patients received HAART including PI: indinavir in 35 cases, ritonavir in 3, and nelfinavir in 2. The first NRTI used was d4T in 35 cases and zidovudine in 5; the second NRTI used was lamivudine in 32 patients, didanosine in 7, and zalcitabine in 1 case. During the observation period, 3 patients switched from didanosine to lamivudine.

Before the introduction of d4T, all the patients were treated with zidovudine, but only 5 of them continued this drug; we therefore considered, for our analysis, a first group (Group 1) which included all the 40 patients – 5 of whom were never treated with d4T but with zidovudine – and a second group (Group 2) of 35 patients who were treated with both PI and d4T.

The total treatment duration between the first and the second DEXA was not the same for all the patients (Table I).

Cardiovascular evaluation was performed in all the patients and included an ECG every 6 months and a color Doppler echocardiography plus a color Doppler ultrasound of the carotid vessels (AU5-EPi Esaote SpA, 75 MHz probes) carried out in close chronological sequence with the second DEXA. Male patients with breast enlargement were also submitted to breast ultrasound. Two years after the last DEXA examination, 38/40 patients are still under observation, while one patient died of a liver carcinoma and another one was lost to follow-up.

Seven female patients are still under treatment with PI + d4T, 3 are being treated with PI but not d4T, and 1 with d4T but not PI; 19 males are under treatment with both PI + d4T, 2 are being treated with PI but not d4T, 5 with d4T but not PI, and 1 is not being treated with either of these drugs.

**Statistical analysis**

The statistical analysis (Statgraphics Plus-Manugistics Inc., Rockville, MD, USA) was performed using the Student’s t-test for paired differences, the Wilcoxon test, and simple and multiple linear regression analysis. This last analysis was carried out to evaluate the relationship of the changes in fat mass or LBM – expressed as percentage differences (PD) with the first DEXA examination – and the duration (months) of treatment with PI, d4T and MD.

**Results**

**Immunohematological tests**

In the overall evaluation of our 40 patients (Group 1) there was a significant increase in total cholesterol, a non-significant increase in triglycerides, and a decrease in HDL cholesterol. This last finding was less evident in Group 2. The serum levels of amylase decreased slightly. Hyperglycemia was recorded in 1 case only. There was a marked increase in hemoglobin levels.

<table>
<thead>
<tr>
<th>Table I. Total treatment duration in Groups 1 and 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 40)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>MD (months)</td>
</tr>
<tr>
<td>PI</td>
</tr>
<tr>
<td>d4T</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD (range values in brackets). d4T = stavudine; MD = months between the first and the second dual-energy X-ray absorptiometry; PI = protease inhibitors.
There was an evident increase in the number of CD4 T-cells but this was less evident for the CD8 T-cell count (Table II).

Fat and lean tissues

The fat and lean weight variations of each body region are reported in Table III.

The peripheral fat tissue was expressed as the sum of the fat in each of the four limbs. Figure 1 shows our results: 13 patients presented with fat loss, 7 with fat accumulation, and 20 with a combination of both.

### Table II. Descriptive statistics and paired differences in Groups 1 and 2.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n = 40)</th>
<th>Group 2 (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>t</td>
</tr>
<tr>
<td>CD4 (cells/mm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>257.75 ± 172.15</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>423.83 ± 209.39</td>
<td>5.875</td>
</tr>
<tr>
<td>CD8 (cells/mm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>786.73 ± 405.59</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>879.43 ± 359.71</td>
<td>1.421</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>183.95 ± 53.62</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>203.83 ± 51.41</td>
<td>2.758</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>40.91 ± 11.22</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>36.30 ± 10.22</td>
<td>-2.246</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>197.85 ± 145.77</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>211.53 ± 142.38</td>
<td>0.510</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>169.05 ± 75.37</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>158.58 ± 62.84</td>
<td>-1.225</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time I</td>
<td>13.71 ± 1.62</td>
<td></td>
</tr>
<tr>
<td>Time II</td>
<td>14.33 ± 1.62</td>
<td>2.931</td>
</tr>
</tbody>
</table>

### Table III. Differences between the first and the second dual-energy X-ray absorptiometry.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n = 40)</th>
<th>t</th>
<th>df</th>
<th>p</th>
<th>Group 2 (n = 35)</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limb fat</td>
<td>-683.33</td>
<td>-3.275</td>
<td>0.0022</td>
<td>-746.71</td>
<td>-3.17</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limb fat</td>
<td>-138</td>
<td>-1.624</td>
<td>0.1100</td>
<td>-143.6</td>
<td>-1.48</td>
<td>0.150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk fat</td>
<td>483.68</td>
<td>1.830</td>
<td>0.0740</td>
<td>543.89</td>
<td>1.93</td>
<td>0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limb LBM</td>
<td>424.43</td>
<td>2.76</td>
<td>0.0088</td>
<td>468.89</td>
<td>2.84</td>
<td>0.0075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper limb LBM</td>
<td>-265.23</td>
<td>-2.21</td>
<td>0.033</td>
<td>-325.74</td>
<td>-2.8</td>
<td>0.0082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trunk LBM</td>
<td>858.33</td>
<td>3.66</td>
<td>0.00075</td>
<td>868.51</td>
<td>3.53</td>
<td>0.0012</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LBM = lean body mass.
the following fitted model: PD the of the lower limb fat 52.2271 - 2.97928 * PI + 1.05816 * d4T - 0.278834 * MD.

This results in a confirmation of both the influence of PI – the angular coefficient of which is negatively more emphasized as compared with Group 1 – and the lesser influences of MD and d4T, which, in fact, were not significant (p > 0.05).

In Group 2 (without the 5 cases in whom d4T was not administered), the mean fat mass decreased to an even greater degree, although, paradoxically, d4T tended to slightly restore the fat mass of the lower limbs; the influence of MD was still irrelevant.

In conclusion, since d4T and MD were both not significant, they were removed from the model. The final equation, therefore, was PD of the lower limb fat 63.5203 - 2.56868 * PI, a very similar equation to that obtained for the 40 patients.

Finally, considering the response variability of the patients, we have limited the analysis to the 23 patients who presented a decrease in the lower limb fat and were treated with both PI and d4T (Table IV).

Multiple regression analysis confirmed that PI is the only significant variable; the simple linear regression with this variable, therefore, was the following: PD of the lower limb fat 12.0837 - 1.53298 * PI.

**Upper limb fat**

The differences in the decrease of upper limb fat expressed in grams – less evident as compared with the lower limb fat – were not significant in both groups (Table III).

In the 23 patients with a fat decrease treated with both PI and d4T, the mean of the upper limb fat difference was -304.96, t = -2.55, p = 0.018. Multiple and simple linear regression percent difference analyses did not reveal any significance of the three variables (PI, d4T, MD).

**Trunk fat**

The analysis identified a slight increase, both absolute (Table III) and in percentage, of the trunk fat, but it should be stressed that DEXA is unable to reliably distinguish between the subcutaneous and visceral fat of the thorax and abdomen; both anatomical regions were therefore considered as “trunk”19,20.

The mean of the weight differences of the trunk fat was not significant in both Groups 1 and 2 (Table III).

**Lean body mass**

Table III shows the mean LBM weight differences in the various body districts.

Multiple regression analysis of the LBM performed in the 35 cases in whom both d4T and PI were administered did not reveal any significant influence of d4T. The analysis was therefore carried out on all the 40 cases, with the following PD:

- PD of the lower limb LBM mean 2.93 ± 6.74;
- PD of the upper limb LBM mean -2.87 ± 12.35;
- PD of the trunk LBM mean 3.64 ± 6.27.

Multiple regression analysis yielded significant results only for the PD of the upper limb LBM. These were related to the duration of PI treatment. The equation of the fitted model was: PD of the upper limb LBM -26.9609 + 0.785984 * PI.

### TABLE IV. Descriptive analysis of 23 patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD of LF*</td>
<td>-40.28 ± 19.01</td>
<td>-71.64</td>
<td>-4.98</td>
</tr>
<tr>
<td>PI</td>
<td>32.70 ± 6.79</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>d4T</td>
<td>29.65 ± 8.28</td>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>MD</td>
<td>28.35 ± 13.12</td>
<td>12</td>
<td>45</td>
</tr>
</tbody>
</table>

d4T = stavudine; LF = lower limb fat; MD = months between the first and the second dual-energy X-ray absorptiometry examinations; PD = percentage difference; PI = protease inhibitors.

* PD of the fat tissue evaluated between the first and the second dual-energy X-ray absorptiometry.
Bone mineral density

Bone mineral density of the 40 patients evaluated at L2, L3, and L4 levels, was:

- first DEXA mean 0.99 ± 0.13 (g/cm²);
- second DEXA mean 0.97 ± 0.14 (g/cm²).

The Wilcoxon test, considering the distribution asymmetry, was not significant: mean bone mineral density differences - 0.024 ± 0.06, W = 1.77, p = 0.78.

Cardiovascular examination

None of our patients but one showed any abnormalities suggestive of coronary heart disease at both electrocardiography and echocardiography. A Doppler ultrasound of the epiaortic vessels performed in 32 cases matched with the same exam of other patients treated with NRTI, of those with or without NRTI, and of patients of the same age present in our database. There were no significant differences and the median of the carotid intima-media wall thickness was ≤ 1.2 mm.

After 2 years of observation including clinical examinations and the patients’ self-reported questionnaires (changes in the body morphology, such as thinning of the limbs and buttocks, abdomen fat gain, sunken cheeks and/or temples, breast enlargement in women), the following findings were found in 38/40 patients:

1) modest habitus changes in 8 men (5 d4T + PI, 1 d4T, 1 PI, 1 without d4T and PI) and in 2 women (1 d4T + PI, 1 PI);
2) marked body habitus changes (facial and lower limb fat loss in particular) in 19 men (14 d4T + PI, 4 d4T, 1 PI) and in 9 women (6 d4T + PI, 1 d4T, 2 PI).

Discussion

The body fat redistribution syndrome in HAART recipients was first described in 1997, but only in 1998 was this finding more extensively studied. Both PI and NRTI (above all d4T) contribute to HIV-associated lipodystrophy syndrome; the influence of these two classes of drugs is however still under debate.

In the present study, we evaluated 40 HIV-infected patients who were divided into two groups: Group 1 which included all the 40 patients, 5 treated without d4T but with zidovudine, and Group 2 which included 35 patients, all treated with PI and d4T at the same time. In all of them, the lipid abnormalities observed were related to an increase in the total cholesterol values and a decrease in the HDL cholesterol values (this latter finding was statistically significant in Group 1 only). The increase in triglyceride values was not significant in both groups. The levels of amylase were normal in all the patients studied. Hyperglycemia was recorded in only 1 patient with a trend towards diabetic. The increase in the hemoglobin and CD4 T-cell count and the decrease in HIV-RNA were both significant findings. The viral load was < 400 cps/mL in nearly all the patients (data not shown).

The modifications in the adipose tissue of the various body districts were not homogeneous; according to the Marrakech classification, our 40 patients could be included in the first three categories: 13 were type 1 (fat loss), 7 were type 2 (fat accumulation), and the remaining 20 were type 3 (combined form).

Multiple regression analysis indicated that the absolute and percentage decrease of the lower limb fat was due to PI, while the influence of d4T and MD seems irrelevant.

As an unusual finding, a slight, although not significant, tendency of d4T to restore the lower limb fat was noted in the second group of 35 patients.

It is however evident that the patients cannot be easily evaluated by one single calculation: the variability of the results, in fact, remarkably influenced the survey. In a more homogeneous sample (23/26 patients treated with PI and d4T, all of them with a decrease in the lower limb adipose tissue), again the only significant variable factor was PI. Similarly, the interpretation of the decrease of the upper limb fat – as an absolute variation expressed in grams and PD – was disappointing. The whole model was not satisfying and the variability was considerable. In none of these three groups of patients (40, 35, and 23 patients) was it in fact possible to appreciate any incisiveness of the variables.

The increase in the trunk volume, even when considering the reservations due to the technique used, was not statistically significant in both groups, neither as an absolute nor as a percentage value.

The LBM had increased in the lower limbs and the trunk, but had decreased in the upper limbs; PI was the active variable in relation to the duration of administration.

The reduced bone density recorded in HIV-infected patients has been associated either with the dyslipidemia secondary to the use of PI, or with the lactic acidemia secondary to the mitochondrial toxicity due to the use of NRTI. Vascular osteonecrosis has also been described in our 40 patients, all of them treated with long-term NRTI, the bone mineral density decreased, but the difference was not significant. In 3 male patients, physical examination and ultrasound were suggestive of gynecomastia, i.e. bilateral enlargement of the breast tissue due to hyperplasia of the mammary glands in the absence of adipose tissue masses. It should however be noted that, unlike other cases described in the literature, we did not administer ritonavir to our patients.
After the first descriptions of premature coronary artery disease in patients treated with PI, an increase in the number of cases of coronary diseases, myocardial infarctions, and of an augmented carotid intima-media wall thickness has been recorded during the last 3 years in HAART patients and the length of the treatment was included among the various risk factors.

Among our patients, only one had a myocardial infarction during treatment, but he had a positive family history and was a heavy smoker (> 40 cigarettes per day). In the remaining 39 patients, all of them followed up by means of electrocardiography, echocardiography, and color Doppler ultrasound of the epiarterial vessels every 6 months, no significant cardiac anomalies were reported. However, our observations were made during a shorter period than is usually required for the development of serious cardiovascular diseases. Data from larger cohorts, ideally prospectively collected, are clearly needed.

The influence of HAART on cardiovascular diseases should be confirmed: approximately 500,000 individuals in western countries are presently receiving antiretroviral treatments without a higher incidence of coronary disease, transient ischemic attack, RIND, or stroke. We therefore believe that not only these antiretroviral therapies but also the family history and lifestyle (including smoking) should be considered together with a longer follow-up for the evaluation of the cardiovascular risk.

In 28/38 of our patients, after many years of treatment, we found peripheral fat loss, especially of the face and lower limbs, more severe in women, but the fact that NRTI and PI may have independent and interacting effects on the development of lipodystrophy does not necessarily mean that these drugs are the only factors responsible for these changes. We believe that the subcutaneous adipose tissue loss, manifesting as sunken cheeks and lower limb thinning, is an HIV-related phenomenon. In a previous paper, we had noted how 23/25 HIV-infected patients had significant limb thinning even before treatment with d4T and PI.

The present study is burdened by some biases which must be taken into account: the first one is the small number of patients of different ages. The second point is that the subcutaneous adipose tissue loss, manifesting as sunken cheeks and lower limb thinning, is an HIV-related phenomenon. In a previous paper, we had noted how 23/25 HIV-infected patients had significant limb thinning even before treatment with d4T and PI.

The third aspect to be considered is that d4T and PI were not administered for the same length of time and that our patients had a long NRTI exposure, especially zidovudine. Finally, while estimates vary greatly (from 10 to 75% of HAART patients develop a lipodystrophy approximately 10 months after the initiation of combined treatment), our patients received HAART for at least 12 months and had the same time interval between the first and the second DEXA examination.

**Riassunto**

Scopo dello studio è stato valutare l’influenza di tre variabili – inibitori delle proteasi (PI), inibitori nucleosidici della transcriptasi inversa (stavudina) e periodo di terapia combinata – nell’indurre modificazioni della distribuzione somatica di tessuto adiposo e muscolare, diminuzione della densità minerale ossea, alterazioni metaboliche e malattie cardiovascolari in pazienti trattati con terapia antiretrovirale (HAART) per l’infezione da virus dell’immunodeficienza umana (HIV).

Quaranta pazienti (29 uomini, 11 donne, età media 39,13 ± 7,82 anni, range 28-61 anni) trattati con HAART per un periodo di 12-43 mesi sono stati arruolati e monitorati per le variazioni di tessuto adiposo e muscolare, densità minerale ossea, modificazioni metaboliche e complicanze cardiovascolari. Le differenze ponderali dei tessuti grassi e magri, la densità minerale ossea sono state valutate con esame DEXA (dual energy X-ray absorptiometry) all’inizio e alla fine del periodo di terapia. Esami elettrocardiografici ogni 6 mesi. Ecco cardiogramma color Doppler ed eco color Doppler dei vasi epiarterici praticati in concomitanza della seconda DEXA. Vengono riportati i parametri immunoematologici all’inizio e alla fine dello studio.

Al termine dell’osservazione 13 pazienti mostravano globalmente perdite di tessuto adiposo, 7 accumulo e 20 quadri misti. All’esame distrettuale, la diminuzione significativa del tessuto adiposo degli arti inferiori era attribuibile a PI, nessuna delle tre variabili appariva collegata con quella degli arti superiori. La diminuzione modesta del tessuto muscolare degli arti superiori era secondaria all’impiego di PI. Non sono state dimostrate alterazioni cardiovascolari. Di scarso rilievo la diminuzione della densità minerale ossea.

In conclusione nei 40 pazienti studiati l’unica variazione significativa era la diminuzione del tessuto adiposo degli arti inferiori, attribuibile secondo la nostra interpretazione, a PI. L’impiego della HAART, nel periodo di osservazione, non modificava significativamente la massa minnerale ossea né induciva complicanze cardiovascolari.

**Parole chiave:** Lipodistrofia; Osteopenia; Rischio cardiovascolare; Terapia antiretrovirale.
References


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