SUMMARY - This study assessed glucose tolerance, insulin sensitivity and lipid parameters in HIV-infected patients presenting with lipodystrophy during HAART including protease inhibitors. Fourteen consecutive patients from Rothschild Hospital treated with HAART and presenting with marked facial lipatrophy were evaluated. A 75 g oral glucose tolerance test (OGTT) with measurement of plasma glucose, insulin, proinsulin and free fatty acids at T0, 30, 60, 90 and 120 min was performed. Lipid parameters (triglycerides, cholesterol, apolipoproteins A1 and B) were studied as well as nutritional and inflammatory markers (albumin, prealbumin, transferrin, haptoglobin, orosomucoid, C-reactive protein), endocrine and cytokine parameters (thyrotropin, cortisol, leptin, interleukin-6, HIV viral load and CD4-lymphocyte count). These patients were compared with 20 non-lipodystrophic protease inhibitor-treated patients. The measurements performed during OGTT showed that among the 14 lipodystrophic patients, 11 (79 %) presented with diabetes (5 patients) or normal glucose tolerance but with insulin resistance (6 patients). This frequency was strikingly different in the group of non-lipodystrophic patients, which included only 4 (20 %) presenting with diabetes (1 patient), or impaired glucose tolerance (2 patients), or normal glucose tolerance but with insulin resistance (1 patient). Hypertriglyceridaemia was present in 11 lipodystrophic (79 %) versus 7 non-lipodystrophic patients (35 %). Nutritional and endocrine measurements were normal. An abnormal processing of proinsulin to insulin was excluded. Thus, lipodystrophy during HAART was associated with diabetes, insulin resistance and hypertriglyceridaemia. Diabetes, diagnosed by basal and/or 120 min-OGTT glycaemia, seems more frequent than previously described. The therapeutic consequences of these results deserve evaluation in clinical trials.

Key-words: protease inhibitors, lipodystrophy, insulin resistance, diabetes, hypertriglyceridaemia.

RÉSUMÉ - Diabète, insulino-résistance et dyslipidémie chez des patients VIH + présentant un syndrome lipodystrophique sous traitement antirétroviral intensif comportant des antiprotéases. Cette étude se proposait d'évaluer la tolérance au glucose, la sensibilité à l'insuline et les paramètres lipidiques chez 14 patients VIH + présentant un syndrome lipodystrophique sous traitement antirétroviral intensif comportant des antiprotéases. Ces patients, suivis à l'hôpital Rothschild, et présentant une lipatrophie marquée au niveau du visage, ont été étudiés sur le plan clinique et métabolique. Nous avons réalisé une hyperglycémie provoquée par voie orale (HGPO) avec des dosages répétés sur deux heures de glycémie, insuline, pro-insuline et acides gras libres. Nous avons également mesuré des paramètres lipidiques (triglycerides, cholestérol, apolipoprotéines A1 et B), nutritionnels et inflammatoires (albumine, préalbumine, transferrine, haptoglobine, orosomucoïde, protéine C réactive), certaines hormones et cytokines (TSH, cortisol, leptine, interleukine 6), ainsi que la charge virale et le taux des lymphocytes CD4. Un groupe contrôle de 20 patients sous traitement antirétroviral intensif comportant des antiprotéases mais non lipodystrophiques a été évalué en parallèle. Chez les 14 patients lipodystrophiques, les résultats de l'HGPO ont montré que 11 d'entre eux (79 %) présentaient soit un diabète (5 patients), soit une tolérance au glucose normale mais une résistance à l'insuline (6 patients). Cette fréquence était significativement différente de celle obtenue chez les 20 patients non lipodystrophiques : seulement 4 (20 %) présentaient soit un diabète (1 patient), soit une altération de la tolérance au glucose (2 patients), soit une tolérance au glucose normale mais une insulino-résistance (1 patient). Une hypertriglycéridémie était présente chez 11 des 14 patients lipodystrophiques (79 %) versus 7 des 20 patients non lipodystrophiques (35 %). Les marqueurs nutritionnels et hormonaux étaient normaux. Un trouble de la maturation de la pro-insuline en insuline a été exclu. Ces résultats montrent que le syndrome lipodystrophique sous thérapie comportant des antiprotéases est fréquemment associé avec un diabète, une résistance à l'insuline et une hypertriglycéridémie. Le diabète, diagnostiqué sur les glycémies à jeun et/ou à 120 minutes d'une HGPO, est plus fréquent que précédemment rapporté. Les conséquences thérapeutiques de ces résultats doivent être évaluées par des études cliniques.

Mots-clés : antiprotéases, lipodystrophie, insulino-résistance, diabète, hypertriglycéridémie.

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Highly active antiretroviral therapy (HAART) including protease inhibitors has dramatically improved morbidity and mortality rates in HIV-infected patients [1]. However, the long-term side-effects of such treatment are largely unknown. The appearance of alterations in body fat distribution in some HIV-infected patients has recently been described as benign symmetric lipomatosis [2], accumulation of trunk fat [3] or visceral abdominal fat [4], or peripheral lipodystrophy sparing the abdomen [5, 6]. Association with protease inhibitor therapy was reported in most cases. However, Lo et al. [3] described 8 cases of “buffalo hump” in men with an HIV infection, 4 of whom were not treated with these drugs. Recently, insulin sensitivity was found to be decreased in protease inhibitor-treated patients [7], but the presence of lipodystrophic signs was not indicated in this study. Two reports suggested the presence of insulin resistance in lipodystrophic patients, who were, however, only poorly explored at the individual level [5, 6]. During HAART, diabetes, associated or not with lipodystrophy, has been found to be rare [5, 6, 8] and often related to a family history. However, since diagnosis was based on fasting glycaemia, and since glucose tolerance tests were rarely performed, the occurrence of diabetes could have been underestimated.

Generalised or partial lipodystrophies have been described in very rare syndromes without HIV infection, in a context of familial transmission. The molecular causes of these genetic diseases are presently unknown [9]. As major insulin resistance with progressive defects in glucose tolerance has been observed in such patients [10], we decided to conduct a careful study of those presenting with lipodystrophy during HAART including protease inhibitors and to evaluate their glucose tolerance and insulin sensitivity, together with different endocrine and lipid parameters.

This study showed that a high proportion of the 14 lipodystrophic, protease inhibitor-treated patients exhibited abnormalities in glucose tolerance and/or insulin sensitivity and in hypertriglyceridaemia. These results were strikingly different from those obtained in a group of 20 non-lipodystrophic, protease inhibitor-treated patients. We suggest that blood glucose and serum lipid parameters should be carefully determined before and during HAART, in particular in patients with lipodystrophy, in order to treat metabolic disturbances. The therapeutic consequences of these results deserve further clinical study.

**Patients and Methods**

We studied the physical and biological characteristics of consecutive HIV-infected patients treated at Rothschild Hospital with HAART including protease inhibitors and presenting either with a clinically marked facial lipodystrophy (14 patients) or without any lipodystrophic signs, *i.e.* peripheral lipodystrophy or central increased adiposity (20 patients).

Clinical examination of all patients was carried out by the same physician (T-H. N), using a standardised procedure, and included measurements of weight, height and blood pressure. Waist and hip girths were measured with an inelastic tape at the umbilicus and the iliac crests. Collected data concerned information about familial and personal antecedents of diabetes, duration of HIV infection, time of onset, and duration of treatments.

In the lipodystrophic group of patients, biochemical parameters measured under fasting conditions were plasma electrolytes, creatinine, hepatic enzymes (alanine aminotransferase, aspartate aminotransferase), plasma protein profile (albumin, prealbumin, transferrin, C3, haptoglobin, orosomucoid, C-reactive protein), triglycerides, cholesterol, apolipoproteins A1 and B, and thyrotropin. Plasma cortisol (RIA, Immunotech, France) was assessed at 8 a.m., before and after an overnight low dose dexamethasone suppression test. A 75 g oral glucose tolerance test (OGTT) was performed after an overnight 12 h fast, and plasma glucose, specific insulin with no cross-reactivity with proinsulin (IMX, Abbott, USA), total (intact and split) proinsulin (IRMA using two monoclonal antibodies, [11]), and free fatty acids (NEFA C, Wako, Oxoid, France) were determined at 0, 30, 60, 90 and 120 min. Leptin (RIA, HL81K, Linco Research, USA), interleukin-6 (IL-6, KIC 1261, IRMA, Medgenix, Belgium), and CD4-lymphocyte counts and HIV viral load (QuantiPlex HIV-1, Chiron, USA) were assessed immediately before the onset of protease inhibitor therapy and at the time of examination.

Statistical analyses were performed using the non-parametric Kruskal-Wallis test for comparisons between the three subgroups, which were defined according to the results of the OGTT, and the Mann-Whitney rank-sum test to compare the parameters between two subgroups within the three [12]. The Wilcoxon matched-pairs signed-rank test was used to compare IL-6, leptin, CD4-lymphocyte and HIV RNA levels before and during protease inhibitor therapy [12]. Correlations between triglyceridaemia and free fatty acid (FFA) values were assessed using the Spearman test [12]. A two-tailed *p* value of less than 0.05 was the criterion for statistical significance. For statistical analyses, the lower limit of detection value was assigned as a measurement when the value was under this lower limit (IL6 and HIV viral load).

For the clinically defined control group of non-lipodystrophic protease inhibitor-treated patients, standard biochemical parameters (plasma electrolytes, creatinine, hepatic enzymes), the fasting lipid profile (triglycerides, cholesterol, apolipoproteins A1 and B), and plasma glucose and insulin were determined at 0 and 120 min of a 75 g OGTT.

RESULTS

From April 1996 to May 1998, 1,243 patients were treated at Rothschild Hospital with HAART including protease inhibitors. Fourteen and twenty consecutive patients presenting respectively with marked clinically defined lipodystrophy or without any lipodystrophic signs were studied here.

Clinical characteristics

The clinical characteristics of lipodystrophic patients are summarised in Table I: 4 women and 10 men (mean age 49 years, range 33-73) known to be HIV-infected for a mean 11 years (range 5-14). All patients were treated with HAART associating protease inhibitors and nucleoside analogues for a mean 19.5 months (range 11-27). They were all treated with indinavir at the onset of lipodystrophy, and 2 patients were treated with ritonavir and/or saquinavir before or after indinavir therapy. The mean time between the onset of protease inhibitor therapy and the appearance of lipodystrophy was 5.7 months (range 2-18). No patient was taking glucocorticoids, anabolic hormones, oral contraceptives, pentamidine, thiazides, or ddl, or had any family history of abnormal fat distribution. All presented with marked facial lipodystrophy and, in some cases, cachectic features leading to psychological and social difficulties. Lipodystrophy also concerned upper, lower or all limbs in 12 patients. The lack of adipose tissue resulted in prominent veins and muscular features. Lipodystrophy was associated with fat accumulation involving the dorsocervical pad (“buffalo hump”) in 4 male patients, the abdominal region in 9 cases and the breasts in all female patients (Table I). No other signs of Cushing’s syndrome were found. Therefore, in 13 out of 14 patients, lipodystrophy was associated with peripheral lipodystrophy and central adipose tissue excess (Table I). The waist to hip girth ratio was of android type (i.e. above 0.88) for all women, with a mean value of 1.01. Three out of four women suffered from hyperandrogenic signs (hirsutism, alopecia and/or acne), which appeared concomitantly to lipodystrophy without marked alterations in their basal plasma androgen values (data not shown). Body wei-

Table I. Clinical characteristics of the 14 patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
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<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Age (year)</td>
<td>38</td>
<td>33</td>
<td>36</td>
<td>34</td>
<td>37</td>
<td>43</td>
<td>44</td>
<td>54</td>
<td>53</td>
<td>54</td>
<td>62</td>
<td>73</td>
<td>62</td>
<td>58</td>
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<tr>
<td>Familial history of diabetes</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Duration of HIV infection (year)</td>
<td>11.2</td>
<td>10.6</td>
<td>11.3</td>
<td>11.9</td>
<td>5.3</td>
<td>12.9</td>
<td>11.3</td>
<td>13.3</td>
<td>12.6</td>
<td>11.8</td>
<td>7.8</td>
<td>10.6</td>
<td>14</td>
<td>7.8</td>
</tr>
<tr>
<td>Type of protease inhibitor treatment</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>ritonavir (2 months) then indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
<td>indinavir</td>
</tr>
<tr>
<td>Duration of protease inhibitor therapy (months)</td>
<td>20</td>
<td>11</td>
<td>11</td>
<td>24</td>
<td>22</td>
<td>27</td>
<td>16</td>
<td>17</td>
<td>19</td>
<td>23</td>
<td>22</td>
<td>21</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Time between protease inhibitor therapy and lipodystrophy (months)</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>18</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>15</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>BM (kg/m²)</td>
<td>23.2</td>
<td>21.1</td>
<td>19.7</td>
<td>20.7</td>
<td>23.5</td>
<td>23.1</td>
<td>27.3</td>
<td>22.4</td>
<td>19.4</td>
<td>21.4</td>
<td>22.1</td>
<td>20</td>
<td>23.8</td>
<td>25.3</td>
</tr>
<tr>
<td>Waist to hip girth ratio</td>
<td>0.98</td>
<td>0.97</td>
<td>1.1</td>
<td>1</td>
<td>0.93</td>
<td>0.86</td>
<td>0.94</td>
<td>1.04</td>
<td>1</td>
<td>0.86</td>
<td>0.98</td>
<td>1.07</td>
<td>1.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Areas of fat accumulation</td>
<td>B, A</td>
<td>B, A</td>
<td>B, A</td>
<td>B, A</td>
<td>N</td>
<td>A</td>
<td>N, T</td>
<td>A</td>
<td>A</td>
<td>N</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

F face
LL lower limbs
UL upper limbs
B breasts
A abdomen
N neck
T thorax
ght was stable since the onset of lipodystrophy for all patients, except one who gained 6 kg and 3 who lost 9 to 11 kg (as the result of a low-calorie diet for diabetes in one case). At the time of examination, body mass indexes (BMI) were in the normal range (19-25) in all cases except one (27.3), with a mean value of 22.4. Blood pressure was normal in all cases except two patients, known to be hypertensive before the onset of lipodystrophy.

Members of the group of 20 non-lipodystrophic patients (5 females, 15 males) were known to be HIV-infected for a mean 7 years (1-13). The duration of HAART associating protease inhibitors (indinavir for 15 patients, nelfinavir for 3, ritonavir for one and an association of ritonavir with saquinavir for one) and nucleoside analogues was a mean 25 months (17-32). Clinically, there were no signs of lipodystrophy, i.e. neither lipoatrophy nor central fat accumulation. Their BMI were in the normal range (mean 21.2; range 18-26). Waist to hip girth ratios were below 0.88 in 4 out of the 5 women, and below 1.0 in 14 out of the 15 men.

**Basal biochemical and endocrine parameters**

In the group of 14 lipodystrophic patients, standard biochemistry (plasma electrolytes, creatinine) did not show any major alterations. Liver enzymes were high in 4 patients, including 2 with chronic C hepatitis and one with a past history of tuberculosis. Plasma protein profiles ruled out the hypothesis of a denutrition state in all patients since albumin, prealbumin and transferrin were not concomitantly decreased. The C3 fraction of complement was not decreased in any patient. Thryrotropin levels were normal in all cases, as cortisol levels for either morning basal levels (195 to 513 nmol/l) or dexamethasone-suppressed levels (24 to 55 nmol/l). Hypertriglyceridaemia (fasting triglycerides above 2 mmol/l) was present in 11 patients, with a mean value of 5.3 ± 3.6 mmol/l, and correlated with fasting FFA plasma values (r = 0.71, p < 0.05). Total cholesterol was normal (3.8 to 6.7 mmol/l) in 10 cases, with a mean value of 5.8 ± 1.8 mmol/l. The apolipoprotein B to A1 ratio was increased in all cases except two (mean ± SD: 1.1 ± 0.3).

In the control group of 20 non-lipodystrophic patients, standard biochemistry was normal. Hypertriglyceridaemia was present in 7 patients, with a mean value of 2.2 ± 2.0 mmol/l. Total cholesterol was normal in 17 patients, with a mean value of 5.6 ± 1.2 mmol/l, but the apolipoprotein B to A1 ratio was increased in 11 cases (mean ± SD: 1.0 ± 0.4).

**Biological variations during protease inhibitor therapy in lipodystrophic patients**

At the time of examination, leptin levels were within the normal range for all patients except one (mean ± SD: 4.1 ± 5.4 ng/ml). When compared to pretreatment levels (mean ± SD: 4.6 ± 3.6 ng/ml), they were not significantly modified, and their variation did not correlate with weight modification. HIV viral loads were decreased from a mean pre-treatment value of 138.3 to a mean 5.9 × 10^4 copies/ml (p = 0.002), and the CD4-lymphocytes counts were impeded in all cases (mean 99 to 269/ml, p = 0.001). IL6 levels were normal except in one patient, and were comparable before protease inhibitor treatment and at the time of examination.

**Metabolic and endocrine parameters during the OGTT (Table II)**

Glucose tolerance was evaluated according to 1998 World Health Organization (WHO) diagnostic criteria [13]. Insulin sensitivity was assessed by four criteria: the homeostasis model assessment parameter (HOMA) [14], insulinaemia at 0 and 120 min [15], and the area under the insulin curve. Furthermore, the extent of the plasma FFA decrease during the test provided indications about sensitivity to the antilipolytic action of insulin. Analysis of these parameters indicated the presence of an insulin resistance state and/or diabetes in 11 out of the 14 lipodystrophic patients. Therefore, three subgroups of patients could be identified.

Subgroup I was composed of patients with normal glycaemic and insulinaemic responses during the OGTT and normal HOMA values, i.e. patients with normal insulin sensitivity. It concerned only three patients (1 to 3), all female (out of four female patients studied). In addition, FFA levels were markedly decreased during the test, indicating a normal sensitivity to insulin. Triglyceridaemia was normal or only slightly increased (Table III), with a mean value of 1.8 mmol/l, significantly lower than in other patients (p = 0.036). In this subgroup, the mean duration of protease inhibitor treatment was 14 months, significantly less than in other patients (mean 21 months, p = 0.05).

Subgroup II was composed of 6 patients (one woman (patient 4) and 5 men (patients 5 to 9) who displayed increased insulinaemia at the basal level and during the OGTT and increased HOMA values without hyperglycaemia. They were therefore considered as normally glucose-tolerant insulin-resistant patients. Hyperinsulinaemia was very striking (above 180 mU/l for 3 patients). In all cases, the 120-min value was above the normal range [15]. Basal FFA levels were normal or increased. Patients 5 and 6, with the highest triglyceride levels, also presented with impaired FFA
decrease during the OGTT, indicating a defective antilipolytic action of insulin. The mean duration of indinavir therapy was 21 months.

The OGTT results led to the classification of 5 patients as diabetic (patients 10 to 14; diabetes being previously unknown in 4 of them). These patients composed subgroup III. Fasting insulinemia was increased in 4 of them, and the levels increased markedly during the OGTT in 2. Patient 10 was slightly hyperinsulinaemic, and the insufficient decrease in FFA level during the OGTT was a further indication of insulin resistance. Patients 13 and 14 (the latter known to be diabetic for 17 months) presented with more severe diabetes, with fasting hyperglycaemia and low insulin secretion. Under these conditions of β-cell failure, insulin resistance could no longer be assessed by insulin values during the OGTT. These patients were markedly hypertriglyceridaemic, with an FFA level either high in the fasting state (patient 13) or not properly reduced during the test (patient 14).

### Table II. Metabolic and endocrine parameters during OGTT in the 14 lipodystrophic patients

<table>
<thead>
<tr>
<th>Subgroup I</th>
<th>Subgroup II</th>
<th>Subgroup III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>1 2 3</td>
<td>4 5 6 7 8 9 10 11 12 13 14</td>
</tr>
<tr>
<td>Time (minutes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 glucose (mmol/l)</td>
<td>5 5.6 5.7</td>
<td>4.5 5.4 5.4 5.6 5.5 5.6</td>
</tr>
<tr>
<td>insulin (mIU/l)</td>
<td>8 10 12</td>
<td>26 20 18 23 51 20</td>
</tr>
<tr>
<td>proinsulin (pmol/l)</td>
<td>10 11 9.5</td>
<td>48 53 68.5 53.5 48 24</td>
</tr>
<tr>
<td>free fatty acids (mmol/l)</td>
<td>0.41 0.95 0.30</td>
<td>0.70 0.58 1.75 0.65 0.91 0.42</td>
</tr>
<tr>
<td>30 glucose (mmol/l)</td>
<td>7.2 8.6 8.8</td>
<td>8.8 5.7 7.8 9.7 10 7.6</td>
</tr>
<tr>
<td>insulin (mIU/l)</td>
<td>53 63 12</td>
<td>235 47 78 282 167 80</td>
</tr>
<tr>
<td>proinsulin (pmol/l)</td>
<td>28.5 31.5 8</td>
<td>136 73.5 101 152 92.5 43</td>
</tr>
<tr>
<td>free fatty acids (mmol/l)</td>
<td>0.29 0.81 0.40</td>
<td>0.40 0.74 2.08 0.52 1.00 0.30</td>
</tr>
<tr>
<td>60 glucose (mmol/l)</td>
<td>7.5 9.3 9.3</td>
<td>8.2 7.7 9.3 9.7 10.3 9.2</td>
</tr>
<tr>
<td>insulin (mIU/l)</td>
<td>63 93 17</td>
<td>261 90 128 539 189 131</td>
</tr>
<tr>
<td>proinsulin (pmol/l)</td>
<td>42 55.5 14</td>
<td>190 123 109 224 107.5 87</td>
</tr>
<tr>
<td>free fatty acids (mmol/l)</td>
<td>0.18 0.34 0.28</td>
<td>0.26 0.51 2.03 0.41 0.66 0.21</td>
</tr>
<tr>
<td>90 glucose (mmol/l)</td>
<td>6.9 5.7 9.4</td>
<td>7.4 7.4 8.6 8.6 7.5 7.9</td>
</tr>
<tr>
<td>insulin (mIU/l)</td>
<td>46 31 26</td>
<td>243 107 112 460 130 122</td>
</tr>
<tr>
<td>proinsulin (pmol/l)</td>
<td>45.5 31 20</td>
<td>243 165 135.5 251 95 102</td>
</tr>
<tr>
<td>free fatty acids (mmol/l)</td>
<td>0.11 0.21 0.22</td>
<td>0.18 0.43 1.79 0.33 1.00 0.64</td>
</tr>
<tr>
<td>120 glucose (mmol/l)</td>
<td>7.0 5.8 7.7</td>
<td>4.8 7.2 7.4 4.8 7.4 6.4</td>
</tr>
<tr>
<td>insulin (mIU/l)</td>
<td>59 37 22</td>
<td>85 86 114 152 93 78</td>
</tr>
<tr>
<td>proinsulin (pmol/l)</td>
<td>57 39 20</td>
<td>68 170 146 156 75 95</td>
</tr>
<tr>
<td>free fatty acids (mmol/l)</td>
<td>0.09 0.16 0.14</td>
<td>0.18 0.4 1.49 0.29 0.36 0.16</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.8 2.5 3</td>
<td>5.2 4.8 4.3 5.7 12.5 5.0</td>
</tr>
<tr>
<td>Insulin area under curve (arbitrary units)</td>
<td>5.9 6.1 2.2</td>
<td>25.5 9.0 11.5 42.2 17.1 11.7</td>
</tr>
</tbody>
</table>

Normal ranges: fasting glycemia: 4.3 - 5.7 mmol/l; fasting insulinemia: 5 -15 mIU/l; HOMA (insulin T0 (mIU/l) x glucose T0 (mmol/l)/22.5): 1-3
This group of diabetic patients was treated with protease inhibitors for a mean duration of 21 months. Table III shows a comparison of triglyceride and cholesterol levels, the duration of protease inhibitor therapy and the different parameters of insulin resistance during the OGTT between the three subgroups of lipodystrophic patients.

Proinsulinaemia values showed good correlation with those of insulinaemia in all patients except patient 14 who displayed hyperproinsulinaemia (a similar situation has already been described during the course of Type 2 diabetes [16]).

The comparison of this group of lipodystrophic patients with the control group of 20 non-lipodystrophic ones revealed striking differences. The latter group was comparable concerning duration of treatment with PI and BMI. With respect to glucose tolerance, one patient was diabetic (fasting glycaemia: 7.2 mmol/l), one glucose-intolerant (120-min OGTT glycaemia: 8 mmol/l), and one had impaired fasting glycaemia (6.1 mmol/l). In addition, a patient with normal glucose tolerance was considered to be insulin-resistant (HOMA of 3.5). The other patients had normal glucose tolerance and insulin sensitivity. Therefore, four non-lipodystrophic patients out of 20 (20 %) had altered glucose tolerance or insulin resistance, versus 11 out of 14 (79 %) lipodystrophic subjects (p < 0.01).

### DISCUSSION

Several studies have recently described alterations in fat distribution in HIV patients treated with HAART including protease inhibitors. However, these lipodystrophies were clinically very diverse. In the present study, patients were at first classified as lipodystrophic in the presence of a clinically marked facial lipoatrophy. Careful clinical examination indicated that limbs were affected by a lack of subcutaneous adipose tissue in 12 out of 14 patients. In addition, central fat accumulation (abdomen, breasts, neck) was observed in 13 cases. Therefore, most patients presented with mixed lipodystrophy (peripheral lipoatrophy and central obesity). When lipodystrophy appeared, all patients were treated with indinavir, since this protease inhibitor was the one most widely prescribed by physicians in our centre.

Carr et al. [6] indicated the presence of insulin resistance in lipodystrophic as compared to non-lipodystrophic protease inhibitor-treated patients on the basis of moderately increased plasma fasting insulin levels (10.1 mU/l versus 7.3) and HOMA values (2.23 versus 1.58). Diabetes was found to be rare in this study (about 2 % of lipodystrophic patients), as also reported by others [8]. However, only basal levels of glucose and insulin were tested. The lipodystrophic group was studied as a whole, without any subclassification. In our study, glucose tolerance and insulin sensitivity were assessed at the individual level by performing an OGTT with measurement of insulinaemia and FFA levels during the test. Several criteria showed an insulin resistance state in 9 of the 12 lipodystrophic patients with preserved insulin secretion: markedly increased HOMA, fasting and 120-min post-stimulative insulin values, increased area under the insulin curve, and a defective decrease in FFA levels during an OGTT. Five patients were found to be diabetic according to WHO 1998 criteria, including 4 whose diabetes was previously unknown. Hyperglycaemia was more severe in 2 patients (13 and 14), both exhibiting marked defective insulin secretion. These data indicate that diabetes in HIV-infected patients on HAART presenting with lipodystrophy is more frequent than previously stated. A family history of diabetes did not appear to predict the occurrence of impaired glucose tolerance in our study. Among 4 patients with such a history, 2 were diabetic (patients 12 and 14) and 2 were not (patients 2 and 9). The frequency of alterations affecting glucose metabolism (altered glucose tolerance or insulin resistance) was markedly increased in lipodystrophic versus non-lipodystrophic protease inhibitor-treated patients. The differences in lipid parameters between the two groups concerned triglyceride levels, which were more elevated in lipodystrophic patients, rather than cholesterol levels, which were comparable in the two groups. Therefore, in patients on HAART including protease inhibitors, altered adipose tissue distribution was associated with resistance to insulin, altered glucose tolerance and hypertriglyceridaemia, as observed in the genetic forms of lipodystrophies.

These multiple cardiovascular risk factors could explain the occurrence of the severe coronary complications already described [17]. In the short follow-up period, patient 10, whose plasma triglyceride level was 11.8 mmol/l at the time of the study, presented acute pancreatitis with triglyceridaemia of 63 mmol/l. Patient 12, aged 73, who was diagnosed as diabetic during this study, presented with hypertension and mixed dyslipidaemia and had a family history of cardiovascular diseases and diabetes, died from myocardial infarction. The direct responsibility of protease inhibitor treatment was difficult to establish.

It is noteworthy that the 3 normally insulin-sensitive lipodystrophic patients in this study were all premenopausal women (among the 4 females investigated in this group). Accordingly, in the non-lipodystrophic group, the 5 studied women, all premenopausal, had normal glucose tolerance and insulin sensitivity and were not hypertriglyceridaemic. This suggests that premenopausal women are relatively protected against insulin resistance related to HAART with protease inhibitors. Since previous reports studied essentially male patients [3, 4, 6, 7], data concer-
ning this syndrome among women are missing, and further study is required.

The pathogenesis of the lipodystrophic syndrome occurring during HAART is unknown. It was not related to drugs known to increase insulin resistance. Cushing’s syndrome was excluded since basal and dexamethasone-suppressed cortisol levels were normal. Abnormal processing of proinsulin to insulin has been suggested [18], as a result of a cross-reactive mechanism in protease inhibitor action. This hypothesis seems very unlikely since proinsulinaemia values were in good correlation with insulinemia values in our patients [15]. In several patients, FFA levels, either high in the fasting state or insufficiently reduced

<table>
<thead>
<tr>
<th>Subgroup I (patients 1-3)</th>
<th>Subgroup II (patients 4-9)</th>
<th>Subgroup III (patients 10-14)</th>
<th>p</th>
<th>p (Subgroups I/II)</th>
<th>p (Subgroups I/III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l) T0</td>
<td>5.4 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>9 ± 5.6</td>
<td>0.025 †</td>
<td>0.429</td>
</tr>
<tr>
<td></td>
<td>6.8 ± 1</td>
<td>6.3 ± 1.2</td>
<td>15.5 ± 8.3</td>
<td>0.010 †</td>
<td>0.603</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.8 ± 0.8</td>
<td>5.2 ± 3</td>
<td>7.4 ± 4.1</td>
<td>0.062</td>
<td>0.121</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.9 ± 1.7</td>
<td>5.8 ± 2.1</td>
<td>5.9 ± 1.8</td>
<td>0.839</td>
<td>0.604</td>
</tr>
<tr>
<td>Duration of protease inhibitor therapy (months)</td>
<td>14 ± 5.2</td>
<td>20.8 ± 4.3</td>
<td>21.2 ± 2.5</td>
<td>0.143</td>
<td>0.120</td>
</tr>
<tr>
<td>Insulin (miU/l) T0</td>
<td>10 ± 2</td>
<td>26 ± 12</td>
<td>28 ± 1</td>
<td>0.022 †</td>
<td>0.020 †</td>
</tr>
<tr>
<td></td>
<td>39 ± 19</td>
<td>101 ± 28</td>
<td>197 ± 120</td>
<td>0.037 †</td>
<td>0.020 †</td>
</tr>
<tr>
<td>Proinsulin (pmol/l) T0</td>
<td>10 ± 1</td>
<td>49 ± 14</td>
<td>43 ± 12</td>
<td>0.041 †</td>
<td>0.020 †</td>
</tr>
<tr>
<td></td>
<td>39 ± 18</td>
<td>118 ± 44</td>
<td>227 ± 81</td>
<td>0.022 †</td>
<td>0.020 †</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.4 ± 0.6</td>
<td>6.3 ± 3.1</td>
<td>7.4 ± 0.6</td>
<td>0.022 †</td>
<td>0.020 †</td>
</tr>
<tr>
<td>Insulin area under the curve (arbitrary units)</td>
<td>4.7 ± 2.2</td>
<td>19.5 ± 12.6</td>
<td>18.1 ± 7.6</td>
<td>0.044 †</td>
<td>0.020 †</td>
</tr>
</tbody>
</table>

† p<0.05
* Patient 13 and 14 were excluded, since their insulin secretion was impaired
All results are expressed as mean ± SD
during the test, could have participated in muscle
insulin resistance, as proposed by Randle et al. [19].
Recently, Carr et al. [20] proposed a role for cytoplas-
mic retinoic-acid binding protein type I and low-
density lipoprotein-receptor-related protein. The bio-
logical activity of these two proteins could be blocked
by protease inhibitors, due to partial homology with
the catalytic region of HIV-I protease. However, these
interesting hypotheses are speculative.

This study indicated that diabetes, insulin resis-
tance and dyslipidaemia are associated with the lipo-
dystrophic syndrome observed with HAART. The
main acute complication of this syndrome is pancrea-
titis related to massive hypertriglyceridaemia. Moreo-
ver, a recent report emphasized the risks of long-term
cardiovascular diseases [17]. Therefore we propose
that, in addition to lipid measurements, fasting and
postprandial glycaemia should be evaluated, in partic-
ular in lipodystrophic patients, in order to manage
diabetic and/or hyperlipidaemic patients. The impact
of nutritional and therapeutic intervention on these
risks deserves evaluation in further studies.

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