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Lipodystrophy in HIV-1 positive subjects is associated with insulin resistance in multiple metabolic pathways

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Introduction

Combination antiretroviral therapy including protease inhibitors for the treatment of HIV-1 infected patients has been associated with the development of a fat redistribution syndrome, which may include both central fat accumulation and peripheral fat wasting [1]. These changes in body fat distribution are frequently accompanied by insulin resistance [2,3]. Insulin resistance is frequently described only in terms of an impairment in the effects of insulin on the uptake of glucose in peripheral tissues. However, insulin influences several metabolic pathways. Insulin increases peripheral uptake of glucose, but decreases endogenous glucose production, the rate of lipolysis and of proteolysis. Insulin sensitivity in HIV related lipodystrophy has only been described in terms of the effects of insulin on peripheral glucose uptake, but not in terms of other effects of insulin.

The aim of the present study was to evaluate the effects of the combination of HIV infection and lipodystrophy on postabsorptive glucose production and insulin sensitivity with respect to the suppressive effects of insulin on endogenous glucose production. Furthermore, we measured oxidative and non-oxidative glucose disposal. In addition we measured plasma free fatty acid (FFA) levels as a reflection of lipolysis.
Subjects and Methods

Subjects

We studied 7 HIV-1 positive men with lipodystrophy (HIV+LD) who were included in the ‘Reverse’ study. This is an ongoing protocol in which HIV-1 infected patients with lipodystrophy are examined for reversibility of the syndrome when replacing the protease inhibitor component in their regimen by the nucleoside reverse transcriptase inhibitor abacavir. Patients eligible for inclusion in this study have to use a protease inhibitor-containing regimen and have a plasma HIV-1 RNA level below 400 copies per millilitre for at least 6 months. Patients with diabetes mellitus, defined as having a fasting glucose concentration above 7.0 mmol/l, were excluded[4].

After 6 weeks of adding abacavir (300mg, two times daily) to their current regimen, patients are randomized to either discontinue their protease inhibitors immediately, or continue protease inhibitor use for another 12 weeks and then stop. Patients referred for this protocol had lipodystrophy in the opinion of their treating physician. Prior to being included this was confirmed by physical examination and by obtaining the patients’ history by two study physicians. Lipodystrophy was defined as the presence of peripheral lipoatrophy, central fat accumulation or both. All assessments reported for the HIV+LD group were performed six weeks after adding abacavir to the current antiretroviral regimen. We included patients who reached this point between February 2000 and November 2000. In the final analysis, one of these seven patients was excluded, because of a technical failure while performing the hyperinsulinaemic euglycaemic glucose clamp.

Six healthy subjects with similar age and BMI were studied as controls. These were also included in another, unrelated dietary intervention study, the results of which have been published elsewhere.[5] The
patients and the controls had used a balanced diet in the weeks before the study.

Both studies were approved by the institutional review board of the Academic Medical Center in Amsterdam. Written informed consent was obtained from all subjects.

**Hyperinsulinaemic euglycaemic clamp protocol (figure 1)**

**Figure 1**

Subjects were admitted to the metabolic clinical research centre and studied in the supine position. Following a 12 hour fast, a catheter was inserted in the antecubital vein of each arm. One catheter was used for sampling of arterialized blood using a heated handbox (60 °C). The other catheter was used for infusion of [6,6-²H₂]-glucose, glucose 20% and insulin. At 09.00 hrs. (t = -2 hr.), after drawing a blood sample for background enrichment of plasma glucose, a continuous infusion of [6,6-²H₂]glucose (>99 % enriched, Cambridge Isotopes, Ma, US) was started at a rate of 0.22 μmol·kg⁻¹·min⁻¹ after a priming dose was administered which equalled 80 minutes of infusion. After 120, 130, 140 and 150
minutes blood samples were drawn for determination of basal endogenous glucose production. FFA concentrations were measured at 120 and 150 minutes. Subsequently at t= + 0,5 hr. a primed continuous infusion of insulin (Actrapid 100 EH/mL, Novo Nordisk Farma B.V., Alphen ad Rijn, The Netherlands) was started for 2,5 hours at a rate of 20 mU·m⁻² body surface area·min⁻¹. Plasma glucose concentration was measured every 5 minutes (Beckman glucose analyzer 2, Palo Alto, CA, US) and glucose 20% was infused at a variable rate to maintain plasma glucose at 5.0 mmol/L. [6,6⁻²H₂] glucose was added to the 20 % glucose solution to achieve glucose enrichments of 2 % to minimize changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose, and thus to allow for accurate quantification of endogenous glucose production [6,7]. The last hour of insulin infusion every 10 minutes blood samples were drawn for determination of endogenous glucose production. FFA concentrations were measured at t= 2,5 hr. and at t=3 hr. During the study subjects were only allowed to drink water.

**Indirect calorimetry**

Oxygen consumption (VO₂) and CO₂ production (VCO₂) were measured by indirect calorimetry using a ventilated hood system (Sensormedics model 2900, Anaheim, Ca). VO₂ and VCO₂ were measured continuously during the final 30 min of both the basal and the hyperinsulinemic periods.

**Body composition**

Body composition was measured with a body impedance analyzer (BIA 109 Akern, Florence, Italy) the morning before the start of the isotope infusion study.
**Analytical procedures**

Plasma insulin concentration were determined by a radioimmuno-assay (Insulin RIA 100, Pharmacia Diagnostic AB, Uppsala, Sweden, intra-assay coefficient of variation (c.v.): 3-5 %, inter-assay c.v.: 6-9 %, detection limit: 15 pmol/L). Serum fatty acids were measured by an enzymatic method (NEFAC; Wako chemicals GmbH, Neuss, Germany, intra-assay c.v. 2-4 %, inter-assay c.v.: 3-6 %, detection limit: 0.02 mmol/L).

**Gas Chromatography–Mass Spectrometry**

Plasma samples for glucose enrichments of [6,6-²H₂]glucose were deproteinized with methanol [8]. The aldonitril penta-acetate derivative of glucose [9] was injected into a gas chromatograph/mass spectrometer system (HP 6890 series II gas chromatograph equipped with a split/splitless injector and an HP 5973 model mass selective detector). Separation was achieved on a J&W DB17 column (30 m · 0.25 mm, df 0.25 μm). Glucose was monitored at m/z 187, m/z 188 and m/z 189. Within each series three control samples with known enrichments were measured for quality control. Glucose enrichments were calculated by dividing the area of the m/z 189 peak by total peak area. Xylose was used as an internal standard to measure glucose concentration.
Calculations and statistics

When endogenous glucose production \( (R_a) \) and glucose disposal \( (R_d) \) are calculated, the added source of labeled glucose entering the system and the exogenous glucose infusate should be taken into account. Thus, \( R_a \) and \( R_d \) were calculated with a modified form of the Steele equations [5]:

\[
R_d(t) = \left\{ \frac{I}{\text{Pct}_p(t)} - \frac{p VG(t) \left[ \frac{d\text{Pct}_p(t)}{dt} \right]}{\text{Pct}_p(t)} + \left[ \frac{\text{Pct}_g}{\text{Pct}_p(t)} x \text{GInf}(t) \right] \right\} - pV \frac{dG(t)}{dt}
\]

and

\[
R_a(t) = \left\{ \frac{I}{\text{Pct}_p(t)} - \frac{p VG(t) \left[ \frac{d\text{Pct}_p(t)}{dt} \right]}{\text{Pct}_p(t)} + \left[ \frac{\text{Pct}_g}{\text{Pct}_p(t)} x \text{GInf}(t) \right] \right\} - \text{GInf}(t)
\]

in which \( I \) represents the constant tracer infusion rate (mg·kg\(^{-1}\)·min\(^{-1}\)), \( t \) time, \( \text{Pct}_p(t) \) the percentage enrichment in plasma glucose taken as the average of 2 consecutive samples, \( p \) the pool fraction, \( V \) the distribution volume of glucose, \( G(t) \) the plasma glucose concentration taken as the average of two consecutive samples, \( d\text{Pct}_p(t)/dt \) the rate of change in the percentage enrichment in plasma (min\(^{-1}\)), \( \text{GInf}(t) \) the rate of infusion of exogenous glucose, \( \text{Pct}_g \) the percent enrichment of the glucose infusate and \( dG(t)/dt \) the rate of change of the plasma glucose concentration. \( pV \) was set at 165 mL/kg. All enrichments were corrected for background enrichments. Reported \( R_a \) and \( R_d \) represent the mean values from 90 to 150 minutes of insulin infusion.

Glucose oxidation was calculated from \( \text{VO2}, \text{VCO2} \) [10]. Non-oxidative glucose disposal was calculated as the difference between total glucose disposal and glucose oxidation.

All data for both HIV+LD and controls are presented as median and interquartile ranges. The differences between HIV+LD subjects and controls were analyzed by the Mann-Whitney test. The differences
within each group between basal measures and those obtained during the hyperinsulinemic euglycemic clamp were analyzed by Wilcoxon’s signed-rank test. We used SPSS (SPSS v.10.0.7 Inc, Chicago) for statistical analysis interpreted at an alpha level of 0.05.
Results

*Patient disposition and baseline characteristics (table 1 and table 2)*

The HIV+LD subjects were comparable to controls with respect to both body mass index, age total body fat and lean body mass (table 1). Median fasting triglyceride concentrations were significantly higher in the HIV+LD group when compared to the healthy controls (3.60 and 0.55 mmol/l, respectively \( p=0.002 \)) All HIV+LD patients had HIV-1 viral loads below 50 copies/ml at the time of assessment. The mean CD4 cell count was 570 (430-755) cells /mm\(^3\). Antiretroviral drug history, details of the regimens used, and body fat changes at enrollment for each of the patients are shown in Table 2.

<table>
<thead>
<tr>
<th><strong>Table 1</strong></th>
<th>Characteristics of HIV positive subjects with lipodystrophy and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>HIV+ LD</strong></td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.9 (21.7-27.0)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>44 (40.8-48.0)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>3.60 (2.34 - 4.51)</td>
</tr>
<tr>
<td>lean body mass (%)</td>
<td>82.6 (80.8-85.4)</td>
</tr>
<tr>
<td>fat mass (%)</td>
<td>17.5 (14.6 -19.2)</td>
</tr>
</tbody>
</table>

BMI: body mass index, ns: not statistically different, yr: year
Lean body mass and fat mass measured by body impedance analysis expressed as percentage from total weight.
Table 2. Antiretroviral treatment (ART) of all six HIV+LD patients, all previous used ART and body fat changes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Current ART Regimen</th>
<th>Months on Current Regimen</th>
<th>Previously Used ART</th>
<th>Body Fat Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AZT/3TC/RTV (100 mg bid)/IDV (800 mg bid)</td>
<td>2</td>
<td>AZT/ddI/IDV</td>
<td>accumulation and atrophy</td>
</tr>
<tr>
<td>2</td>
<td>d4T/3TC/NFV (1250 mg bid)</td>
<td>28</td>
<td>none</td>
<td>accumulation and atrophy</td>
</tr>
<tr>
<td>3</td>
<td>d4T/3TC/RTV (400 mg bid)/SQV (400 mg bid)</td>
<td>37</td>
<td>none</td>
<td>accumulation and atrophy</td>
</tr>
<tr>
<td>4</td>
<td>d4T/3TC/IDV (800 mg tid)</td>
<td>34</td>
<td>none</td>
<td>atrophy only</td>
</tr>
<tr>
<td>5</td>
<td>d4T/3TC/RTV (100 mg bid)/IDV (800 mg bid)</td>
<td>14</td>
<td>AZT/ddC/IDV/NFV</td>
<td>accumulation and atrophy</td>
</tr>
<tr>
<td>6</td>
<td>d4T/3TC/NFV (1250 mg bid)</td>
<td>17</td>
<td>RTV/SQV/IDV</td>
<td>accumulation and atrophy</td>
</tr>
</tbody>
</table>

If not mentioned explicitly standard dosage is being used. bid: two times-daily, tid: three times-daily, qd: one time daily. ZDV: zidovudine 300 mg bid, 3TC: lamivudine 150 mg bid, d4T: stavudine 40 mg bid, ddl: didanosine 400 mg qd, ddC: zalcitabine 0.75 mg bid, RTV: ritonavir, IDV: indinavir, SQV: saquinavir, NFV: nefilnavir. atrophy: peripheral lipoatrophy, accumulation: central fat accumulation.

Table 3. Endogenous glucose production at basal insulin levels and during euglycemic hyperinsulinemic clamp-induced hyperinsulinemia and the relative suppression of endogenous glucose production in HIV+ patients with lipodystrophy (n=6) and HIV- controls

<table>
<thead>
<tr>
<th>Condition</th>
<th>HIV+ lipodystrophy</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal insulin concentration</td>
<td>16.5 (13.5-18.3)</td>
<td>11.2 (10.6-12.0)</td>
<td>0.025</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>8.0 (5.1-11.8)</td>
<td>1.7 (1.4-2.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Suppression of endogenous</td>
<td>53 (61-39)</td>
<td>85 (87-81)</td>
<td>0.004</td>
</tr>
<tr>
<td>glucose production (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as median ± interquartile ranges.
Endogenous glucose production (table 3)

Both fasting plasma glucose and insulin concentrations in the HIV+LD patients compared to controls were not statistically significant different (glucose 5.6 (5.48-5.85) vs. 5.0 (4.95-5.2) mmol/L (p = 0.065); insulin 76.9 (44.1-123.8) vs. 39 (34.3-42.3) pmol/L, p = 0.132). During the hyperinsulinemic euglycemic clamp plasma glucose concentrations were 5.30 (5.21-5.38) and 5.13 (4.98-5.30) mmol/l, and insulin concentrations were 188 (161–205) and 200 (173-211) pmol/L, in patients and controls, respectively (differences not statistically significant).

At basal insulin concentrations, endogenous glucose production was 47% higher in HIV+LD subjects compared to controls (p=0.025). During the hyperinsulinemic euglycemic clamp endogenous glucose production was suppressed to a lesser extent in HIV+LD patients (by 53 (61-39)% in HIV+LD patients versus 85 (87-81)% in the controls, p=0.004).
Peripheral glucose metabolism
(Figure 2)

Peripheral glucose disposal and utilization are shown in Figure 2. At basal insulin concentrations the median total glucose disposal was 16.5 μmol/kg/min and 11.2 μmol/kg/min in HIV+LD and controls, respectively (p = 0.025). The median non-oxidative glucose disposal was 10.8 μmol/kg/min in the HIV+LD group and 4.9 μmol/kg/min in the controls (p = 0.004). When expressing median basal non-oxidative glucose disposal as a percentage of the total disposal this was 71% in the HIV+LD group, compared to 41% in the control group (p = 0.01).

Median insulin-stimulated total glucose disposal was lower in HIV+LD subjects (20.1 μmol/kg/min) when compared to the controls (31.9 μmol/kg/min, p = 0.006).

The hyperinsulinemic clamp increased median total glucose disposal in both groups compared with the basal period, but by only 27% in the HIV+LD group as compared to 201% in controls (p=0.004). Median nonoxidative glucose disposal expressed as a percentage of total disposal was 63% in the HIV+LD group, while this was 62% in the control group (p = 0.997).
**Free fatty acid concentrations**

Plasma fatty acid concentrations were higher in HIV+LD subjects: 0.60 (0.47–0.68) vs. 0.35 (0.28-0.44) mmol/l (p=0.024) at basal insulin concentrations. The reduction of plasma fatty acid concentrations by insulin was less in HIV+LD subjects than in controls: 65 (78-51) vs. 85 (87-84) %, respectively, (p=0.01) resulting in higher plasma fatty acid concentrations in HIV+LD subjects than in controls: 0.17 (0.12-0.35) vs. 0.06 (0.05-0.06) mmol/L, respectively (p=0.004) during insulin infusion.
Discussion

Previous studies have shown that lipodystrophy in HIV infected patients is associated with peripheral insulin resistance using indirect indicators of insulin sensitivity [1-3]. Our results from the euglycemic hyperinsulinemic clamp, which is considered the “gold standard” for assessment of insulin sensitivity, extends the results from these previous studies. Endogenous glucose production was measured during the hyperinsulinemic euglycemic clamp by means of stable isotopes to prevent an underestimation of insulin mediated glucose disposal. Our data show that HIV-1 positive men with lipodystrophy have increased rates of post-absorptive glucose production and lipolysis, which is reflected in higher FFA concentrations. In addition, at insulin concentrations of ~200 pmol/L the endogenous glucose production remained much higher in HIV+LD patients than in controls, in whom the production was suppressed by 85% a value in accordance with data in literature.[11,12]

Insulin stimulated oxidative and non-oxidative glucose disposal were also reduced in HIV+LD, but this probably reflects decreased intracellular glucose availability as no difference between patients and controls was found when these values were expressed as percentage of total glucose disposal. Finally, the ability of insulin to reduce plasma FFA levels was decreased. Taken together, these data indicate that HIV associated lipodystrophy syndrome is characterized by disturbances in multiple pathways of glucose metabolism and in lipolysis, both under basal conditions and during insulin infusion.

The present study compared HIV-1 positive men with lipodystrophy treated with protease inhibitor-containing highly active antiretroviral therapy, and healthy control subjects with a normal body fat distribution. Thus, the groups differed with respect to being HIV-infected, using anti-retroviral therapy as well as the distribution of body fat. We previously demonstrated in the pre-HAART era that insulin
sensitivity in patients with symptomatic HIV infection (without active ongoing opportunistic diseases other than Kaposi's sarcoma and without wasting syndrome) was increased rather than decreased [13,14]. Therefore HIV infection per se does not explain the marked insulin resistance demonstrated in the current study. HIV associated lipodystrophy is clearly associated with insulin resistance, but it remains uncertain whether the reduction in insulin sensitivity is caused directly by certain anti-retroviral drugs and/or by lipodystrophy itself. Nevertheless, both in vitro and in vivo data indicate that certain drugs may have direct effects on glucose metabolism. The protease inhibitor indinavir impairs insulin signalling in HepG2 hepatoma cells [15]. The administration of indinavir during 4 weeks to healthy HIV-negative volunteers resulted in decreased glucose uptake as documented by elevated fasting plasma insulin and glucose concentrations, which were associated with insulin resistance as measured by a hyperinsulinemic euglycaemic clamp [16]. Overt lipodystrophy was not observed in any of these volunteers. This observation, however, does not exclude the possibility, that certain aspects of the lipodystrophy syndrome itself may be involved in the induction of insulin resistance, since clinically overt lipodystrophy is expected to be preceded by alterations in overall lipid metabolism. Our results likewise do not allow to distinguish between the effects on insulin sensitivity of antiretroviral therapy versus those of the lipodystrophy syndrome. Carefully conducted prospective metabolic studies in HIV infected patients initiating HAART may be able to distinguish between the contribution of therapy and changes in lipid metabolism per se, towards the induction of insulin resistance.

Centropetal obesity is a well-known risk factor for the induction of insulin resistance [17]. Our observation of an increased basal glucose production together with a diminished suppressive effect of insulin on this production may be explained by the demonstrated alterations in lipolysis. Fatty acids are known to stimulate glucose production [18]. Fatty acid concentrations were significantly higher in the HIV+LD
patients compared to the controls during basal conditions, and also could be suppressed to a lesser extent during hyperinsulinemia. In lipodystrophy the relative contribution of visceral fat to total body fat mass is increased, as a result of both a reduced peripheral and increased visceral fat mass. This redistribution in fat is likely to be associated with even more pronounced increased fatty acid concentrations in the portal vein of patients with lipodystrophy. It has recently been shown that FFA infusion directly in the portal vein of rats leads to a decreased suppression of endogenous glucose production [19]. Likewise, increased basal glucose production rates and reduced suppression of endogenous glucose production by insulin were demonstrated in healthy, nonobese subjects artificially exposed to elevated plasma fatty acid concentrations [20].

Not all the differences observed between patients and controls in our study can be explained by a difference in plasma fatty acid concentrations. The main determinant of the lower glucose oxidation rate in our patients during the clamp seems to be the diminished stimulation of glucose uptake by insulin. When a correction is made for the differences in glucose uptake between patients and controls, equal rates of glucose oxidation are obtained. This can not be explained by the observed differences in plasma fatty acids, as earlier work by our group has shown that an increase in plasma fatty acids in healthy volunteers specifically inhibits glucose oxidation, but not glucose uptake during an euglycemic clamp [5]. The changes in lipolysis seen in our patients with lipodystrophy cannot simply be an expression of the metabolic changes induced by centropetal obesity. In our patients clear-cut resistance to the effect of insulin on lipolysis is shown, whereas in obese HIV-negative subjects either no resistance [21] or hyperinsulinemia which was sufficient to overcome the resistance[22] has been reported.

In conclusion, lipodystrophy in HIV-infected men exposed to protease inhibitors is associated with both increased endogenous glucose production and resistance to multiple effects of insulin.
References


