Metabolic complications of antiretroviral therapy
van der Valk, M.

Citation for published version (APA):

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Markedly diminished lipolysis and partial restoration of glucose metabolism, without changes in fat distribution following extended discontinuation of protease inhibitors in severe lipodystrophic HIV-1 infected patients

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Introduction

There have been numerous reports on the emergence of metabolic disturbances related to antiretroviral treatment of HIV-1 infection. [1-5] These involve a lipodystrophy syndrome consisting of peripheral fat loss with or without central fat accumulation, hyperlipidemia and disturbances in glucose metabolism. Both nucleoside reverse transcriptase inhibitors (NRTIs) and the protease inhibitors (PIs), have been implicated in the pathogenesis of the syndrome. [1-4, 6-9]

Lipodystrophic HIV-1 infected patients using PIs have severe insulin resistance with respect to the peripheral uptake of glucose. Furthermore, fasting glucose production was increased and there was hepatic insulin resistance with respect to the suppressive effects of insulin on glucose production, compared to both healthy volunteers and to untreated HIV-1 infected patients [10-14]. Administration of a single dose of the PI indinavir to healthy volunteers resulted in an acute, but transient, decrease in insulin sensitivity. [15]

Accordingly, in vitro experiments have undisputedly shown that certain of the PIs impair glucose transport by the inhibition of the intrinsic activity of the glucose transporter GLUT-4. [16, 17] The effect of the withdrawal of PIs on glucose metabolism in HIV-1 infected patients with LD, who have been long-term exposed to PIs has not been precisely delineated. Two small studies using either fasting plasma glucose, insulin or an intravenous insulin tolerance test have reported an improvement [18, 19], whereas another study reported no changes in glucose metabolism [20]. With respect to whole body lipolysis glycerol turnover in patients with lipodystrophy using PIs is increased to the same extent as in untreated HIV-1 infected patients. [12, 21] No studies have yet described the effects of PI withdrawal on lipolysis.

In addition to direct effects of PIs on glucose metabolism maldistribution of fat is likely to have direct effects on glucose
metabolism, as was clearly demonstrated in HIV-1 uninfected patients suffering from congenital lipodystrophy syndromes.[22] Moreover, in a severe lipoatrophic insulin-resistant murine model the subcutaneous implantation of autologous fat resulted in an almost complete reversal of insulin resistance.[23]

Therefore, an important question in HIV-associated lipodystrophy concerns the extent to which PIs per se contribute to the disturbances in glucose metabolism once treatment-induced changes in fat distribution have been established. In order to answer this question, we conducted a prospective study in HIV-1 infected patients with severe lipodystrophy, who, at the time of inclusion, were using PI–based therapy. The different components of glucose metabolism as well as lipolysis were evaluated by hyperinsulinemic glucose clamp both prior and 96 weeks following the replacement of the PIs in their regimen by the NRTI abacavir. Furthermore, any changes in fat distribution were objectified by DEXA and CT scan.
Subjects and Methods

HIV-1 positive men with lipodystrophy were studied, who were included in the ‘Reverse’ study. The main objective of this study was to assess the reversibility of the various components of the lipodystrophy syndrome in HIV-1 infected patients following replacement of the PI-component in patients’ antiretroviral regimens by the nucleoside reverse transcriptase inhibitor abacavir. Patients could be referred for the study if they had lipodystrophy in the opinion of their treating physician. Prior to being included this had to be independently confirmed by two of us (MvdV; PR) based on medical history and physical examination. Lipodystrophy was defined as the presence of peripheral lipoatrophy, central fat accumulation or a combination of both. Furthermore, patients had to use a PI-containing regimen with a plasma HIV-1 RNA level having been 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. [24]

Six weeks after having added abacavir (300mg, two times daily) to their current regimen, patients were randomized to either discontinue their PI immediately, or continue these for another 12 weeks and then stop.

At five timepoints during the course of the study an euglycemic hyperinsulinemic glucose clamp was performed (at the time of randomisation = week 0 and 12, 36, 72 and 96 weeks following the randomisation). Fat distribution was assessed at study entry and at week 96.

The first participant was included in December 1999 and the last reached week 96 in February 2003. All participants used a balanced diet, containing at least 250 gr carbohydrates three days prior to each metabolic study, and were instructed to try to maintain their current weight. The study was approved by the institutional review board of the
Academic Medical Center in Amsterdam. Written informed consent was obtained from all participants prior to study entry.

Hyperinsulinaemic euglycaemic clamp protocol (figure 1)

Subjects were admitted to the metabolic clinical research center 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%, [2H5]-glycerol and insulin. At 09.00 hrs. (t = -2 hr.), after drawing a blood sample for background enrichment of plasma glucose and glycerol, 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 equaled 80 minutes of infusion. At 10.00 hr (t = -1 hr) continuous infusion with [2H5]-glycerol at 0.11 μmol/ kg/min was started after a priming dose of 1.6 μmol/kg. At t = +0, 10, 20 and 30 minutes blood samples were drawn for determination of basal endogenous glucose production and basal glycerol turnover. Subsequently, at t = +30 minutes 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·m² 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. [25,26]. The last hour of insulin infusion every 10 minutes blood samples were drawn for determination of endogenous glucose production and glycerol turnover. During the study subjects were only allowed to drink water.
**Figure 1** Hyperinsulinaemic euglycaemic clamp protocol

![Diagram of the protocol](image)

- **glucose 20% + 2% 6,6-D₂-glucose (variable)**
- **insulin 20 mU/m²/min**
- **D₂-glycerol: bolus 1.6 µmol/kg + continue 0.11 µmol/kg/min**
- **6,6-D₂-glucose: bolus 17.6 µmol/kg + continue 0.22 µmol/kg/min**

<table>
<thead>
<tr>
<th>t = -2</th>
<th>t = -1</th>
<th>t = 0</th>
<th>t = +0.5</th>
<th>t = +2</th>
<th>t = 2.5</th>
<th>t = 3 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

**Plasma collection for isotopes**

**Plasma collection for hormones**

$t = -2$ hrs = 09.00 am; The bars represent continuous infusion from that time onward. 6,6 D₂ glucose: [6,6-²H₂]-glucose; µmol: micromol, kg: kilogram, min: minute D₂-glycerol: [²H₂]-glycerol; µU: milliUnits; m²: square meter; hr: hours

**Indirect calorimetry**

Oxygen consumption ($V O_2$) and CO₂ production ($V CO_2$) were measured by indirect calorimetry using a ventilated hood system (Sensormedics model 2900, Anaheim, Ca). $V O_2$ and $V CO_2$ were measured continuously during the final 30 min of both the basal and the hyperinsulinemic periods.

**Body composition**

Total as well as regional fat mass was quantified in all patients by dual-energy x-ray absorptiometry (Hologic QDR-4500W, software version whole body v8.26A: 5) providing a quantitative assessment of peripheral and truncal fat mass in kg. The ratio between peripheral fat mass, defined as the sum of arm and leg fat, and total fat mass (total fat mass minus head fat) was calculated to adjust for differences in body weight.
A standardized single slice abdominal CT-scan through the level of the fourth lumbar vertebra was performed from which the surface of total (TAT), visceral (VAT) and subcutaneous adipose tissue (SAT) was determined and expressed in square centimeter (cm$^2$). The ratio between SAT: TAT was calculated to assess fat distribution.

**Analytical procedures**

Plasma insulin concentration was 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). Plasma samples for plasma catecholamine concentrations and enrichments of \([6,6\textsuperscript{2}H_2]\)glucose and \([2\textsuperscript{H}_5]\)-glycerol were determined as described before.\[^{10,12}\] Cortisol was determined with a competitive chemiluminescent immuno assay (Immulite, Diagnostic Products Corporation, Los Angeles, USA).

**Calculations**

Endogenous glucose production, total glucose disposal were calculated by non steady state Steele equations as described previously.\[^{10}\] Steele's equation for steady state conditions as adapted for the use of stable isotopes were used to calculate glycerol Rate of appearance (Ra).\[^{12}\]

Glucose oxidation was calculated from VO2, VCO2.\[^{27}\] Non-oxidative glucose disposal was calculated as the difference between total glucose disposal and glucose oxidation. Both glucose oxidation and non-oxidative glucose disposal are expressed as percentage of total glucose disposal to adjust for differences in the total glucose disposal.
Statistical analysis

In a first analysis, the early changes in glucose disposal, endogenous glucose production, and lipolysis following PI withdrawal were evaluated. For this analysis, using Student’s t-test, the changes in these parameters between week 0 and week 12 were compared between the participants randomized to immediate discontinuation of PI and those in whom discontinuation of PI was deferred for 12 weeks. Patients who were discontinued from the study prior to week 12 were excluded from all analyses.

For the main analysis of the long-term effects of PI withdrawal on glucose metabolism, lipolysis and fat distribution all participants were evaluated as their own control after they had discontinued their PI medication. In case of study termination or reinstitution of PI, after week 12, but prior to week 96, a last-value carried forward strategy was applied. For each parameter the difference was calculated between last observation and the time of PI withdrawal. Subsequently, 95% confidence intervals were calculated. Data are presented as means with standard deviations or medians and interquartile ranges where appropriate.
Chapter 8

Results

Patient characteristics at entry and patient disposition

The mean age of the patients included was 47 ± 8 years. The mean weight was 77 ± 15 kg at study entry and did not change significantly over time. All patients had HIV-1 viral loads below 50 copies/ml at the time of study entry. At study entry, the mean CD4 cell count was 509 ± 226 cells/mm³. Antiretroviral drug history, details of the regimens used, and body fat composition at study entry for each of the patients are shown in Table 1.

Table 1 Current and prior antiretroviral treatment (ART) of the nine subjects and changes in body fat at study entry

<table>
<thead>
<tr>
<th>current ART regimen</th>
<th>previously used ART</th>
<th>body fat changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZDV/3TC/RTV (100 mg bid) /IDV (800 mg bid)</td>
<td>2</td>
<td>ZDV/ddI/IDV</td>
</tr>
<tr>
<td>d4T/3TC/NFV (1250 mg bid)</td>
<td>28</td>
<td>none</td>
</tr>
<tr>
<td>d4T/3TC/ RTV (400 mg bid) /SQV (400 mg bid)</td>
<td>37</td>
<td>none</td>
</tr>
<tr>
<td>d4T/3TC/IDV (800 mg bid)</td>
<td>34</td>
<td>none</td>
</tr>
<tr>
<td>d4T/3TC/ RTV (100 mg bid) /IDV (800 mg bid)</td>
<td>14</td>
<td>ZDV/ddC/IDV/ NFV</td>
</tr>
<tr>
<td>d4T/3TC/ NFV (1250 mg bid)</td>
<td>17</td>
<td>RTV/SQV/IDV</td>
</tr>
<tr>
<td>d4T/ 3TC/ IDV</td>
<td>22</td>
<td>ZDV</td>
</tr>
<tr>
<td>ZDV/3TC/ RTV (400 mg bid) /SQV (400 mg bid)</td>
<td>1</td>
<td>d4T</td>
</tr>
<tr>
<td>d4T/3TC/ RTV (100 mg bid) /IDV (800 mg bid)</td>
<td>14</td>
<td>none</td>
</tr>
</tbody>
</table>

If not explicitly mentioned, standard dosage is being used. bid: twice-daily, tid: three times-daily, qd: once 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 tid, RTV: ritonavir, IDV: indinavir, SQV: saquinavir, NFV: nelfinavir. atrophy: peripheral lipoatrophy, accumulation: central fat accumulation
Patient 1 was excluded from the entire analysis because of an increase in plasma HIV-1 RNA 7 weeks after the withdrawal of PI necessitating reinstitution of PI-containing antiretroviral therapy (new regimen d4T/ddI/3TC/RTV/IDV) prior to week 12. Patient 5 had to restart PI containing therapy because of virological failure at week 36 of the study (new regimen ddI/3TC/EFV/LPV/RTV). In both patients, soon after the reintroduction of PI, the plasma HIV-1 RNA load became undetectable.

### Table 2 Patient characteristics while on protease inhibitors (PI) and after PI withdrawal

<table>
<thead>
<tr>
<th></th>
<th>with protease inhibitors (n = 8)</th>
<th>after protease inhibitor withdrawal (n = 8)</th>
<th>absolute change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>77 (15)</td>
<td>78 (16)</td>
<td>+1.8 (-0.3 - +3.8)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>24.0 (4.1)</td>
<td>24.5 (4.1)</td>
<td>+0.5 (-0.1 - +1.1)</td>
</tr>
<tr>
<td>% pt. HIV-1 RNA &lt;50 c/ml</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>CD4-cell count (x10^6/mm^3)</td>
<td>509 (226)</td>
<td>566 (200)</td>
<td>+58 (+11 - +104)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.2 (0.6)</td>
<td>5.3 (0.6)</td>
<td>+0.2 (-0.3 - +0.6)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>6.3 (1.6)</td>
<td>4.6 (1.0)</td>
<td>-1.7 (-2.3 - -1.2)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.92 (0.11)</td>
<td>1.03 (0.32)</td>
<td>+0.10 (-0.1 - +0.3)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.2 (0.78)</td>
<td>2.5 (0.62)</td>
<td>-0.73 (-1.2 - -0.2)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>4.6 (3.0)</td>
<td>2.4 (1.4)</td>
<td>-2.2 (-3.7 - -0.8)</td>
</tr>
<tr>
<td>Basal insulin (pmol/l)</td>
<td>87 (52)</td>
<td>77 (51)</td>
<td>-10 (-34 - +15)</td>
</tr>
<tr>
<td>Insulin during clamp (pmol/l)</td>
<td>224 (95)</td>
<td>238 (87)</td>
<td>+14 (-46 - +75)</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>369 (114)</td>
<td>304 (79)</td>
<td>-64 (-153 - +25)</td>
</tr>
<tr>
<td>Epinephrine (nmol/l)</td>
<td>0.17 (0.11)</td>
<td>0.16 (0.09)</td>
<td>-0.01 (-0.1 - +0.07)</td>
</tr>
<tr>
<td>Norepinephrine (nmol/l)</td>
<td>2.0 (1.3)</td>
<td>1.3 (0.6)</td>
<td>-0.7 (-1.5 - +0.02)</td>
</tr>
<tr>
<td>SAT (cm^2)</td>
<td>79 (20 - 209)</td>
<td>144 (23 - 161)</td>
<td>+10 (-24 - +44)</td>
</tr>
<tr>
<td>VAT (cm^2)</td>
<td>197 (172 - 309)</td>
<td>207 (149 - 234)</td>
<td>-38 (-85 - +10)</td>
</tr>
<tr>
<td>The ratio of SAT over TAT</td>
<td>0.25 (0.1-0.38)</td>
<td>0.41(0.16-0.42)</td>
<td>+0.06(-0.02-+0.14)</td>
</tr>
<tr>
<td>Total amount of peripheral fat in kg</td>
<td>3.7 (2.9)</td>
<td>4.0 (2.4)</td>
<td>+0.24 (-0.55 - +1.0)</td>
</tr>
<tr>
<td>Percentage peripheral fat of total body fat (%)</td>
<td>29.1 (5.8)</td>
<td>29.9 (5.0)</td>
<td>+0.8 (-1.9 - +3.6)</td>
</tr>
</tbody>
</table>

All parameters are expressed as mean (standard deviation) unless otherwise noted.

The absolute changes after protease inhibitor withdrawal are expressed as mean (95% confidence interval). SAT, VAT and the ratio of SAT over VAT are expressed as median and interquartile ranges; n: number of patients; kg: kilograms; BMI: body mass index; m2: square meter; pt. Patients; mm3: cubic millimeter; mmol/l: millimol per liter; pmol/l: picomol per liter; nmol/l: nanomol per liter; SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; TAT: total adipose tissue.
again. Patient 6 died at home, possibly from an acute myocardial infarction, a few weeks after his week 36 study visit. In none of the remaining patients antiretroviral therapy was changed during follow up.

There were no statistically significant changes in basal insulin, glucose, catecholamines and cortisol concentrations over time. In contrast, plasma total-, LDL-cholesterol and triglyceride concentrations had decreased significantly after PI withdrawal. (Table 2)

*Body composition* (Table 2)

The study physician (MvdV) and the patients noted no significant improvements in fat distribution over time. This was confirmed by whole body DEXA scans that showed no improvement either in absolute total peripheral fat mass or peripheral fat expressed as proportion of total fat. In addition, CT scans showed no significant changes in either visceral (VAT) and subcutaneous adipose tissue (SAT) surface area, or in the ratio of SAT over total adipose tissue (TAT).

*Endogenous glucose production* (Figure 2, Table 3)

At study entry the mean fasting glucose production was 16.1 ± 2.5 μmol/kg/min. PI withdrawal resulted in a mean decrease of 1.1 (95%CI - 2.1 - -0.1) μmol/kg/min in fasting glucose production. During the clamp endogenous glucose production was 7.9 ± 2.7 μmol/kg/min at study entry and did not change significantly 96 weeks after PI withdrawal.

At week 12 no differences were observed in the changes in endogenous glucose production both during the clamp and under fasting conditions between the participants randomized to immediate or deferred withdrawal of PI (p = 0.98 and p= 0.55, during the clamp and fasting, respectively).
effects of PI discontinuation on glucose and lipid metabolism

Table 3 Glucose metabolism and lipolysis with protease inhibitors (PI) and after PI withdrawal

<table>
<thead>
<tr>
<th></th>
<th>with Protease inhibitors (n = 8)</th>
<th>after protease inhibitor withdrawal (n = 8)</th>
<th>Absolute change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>fasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- total glucose production (μmol/kg/min)</td>
<td>16.1 (2.5)</td>
<td>15.0 (1.6)</td>
<td>-1.1 (-2.1 - -0.1)</td>
</tr>
<tr>
<td>- percentage glucose oxidation of total glucose disposal (%)</td>
<td>30.5 (10.4)</td>
<td>48.8 (15.6)</td>
<td>18.4 (+4.7 - +32)</td>
</tr>
<tr>
<td>- percentage non-oxidative of total glucose disposal (%)</td>
<td>69.5 (10.4)</td>
<td>51.2 (15.6)</td>
<td>-18.4 (-32.0 - -4.7)</td>
</tr>
<tr>
<td>- glycerol turnover (μmol/kg/min)</td>
<td>2.6 (0.6)</td>
<td>1.8 (0.3)</td>
<td>-0.8 (-1.4 - -0.3)</td>
</tr>
<tr>
<td><strong>clamp</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- total glucose disposal (μmol/kg/min)</td>
<td>21.3 (4.9)</td>
<td>18.4 (5.6)</td>
<td>-3.0 (-6.0 - +0.1)</td>
</tr>
<tr>
<td>- percentage glucose oxidation of total glucose disposal (%)</td>
<td>36.8 (12.7)</td>
<td>48.4 (8.7)</td>
<td>+11.6 (+1.5 - +21.7)</td>
</tr>
<tr>
<td>- percentage non-oxidative of total glucose disposal (%)</td>
<td>63.2 (12.7)</td>
<td>51.6 (8.7)</td>
<td>-11.6 (-21.7 - -1.5)</td>
</tr>
<tr>
<td>- Endogenous glucose production (μmol/kg/min)</td>
<td>7.9 (2.7)</td>
<td>6.9 (1.4)</td>
<td>-1.1 (-2.6 - +0.5)</td>
</tr>
<tr>
<td>- glycerol turnover (μmol/kg/min)</td>
<td>1.8 (0.6)</td>
<td>1.2 (0.5)</td>
<td>-0.6 (-1.2 - -0.1)</td>
</tr>
</tbody>
</table>

All parameters are expressed as mean (standard deviation) unless otherwise noted. The absolute changes after protease inhibitor withdrawal are expressed as mean (95% confidence interval). μmol: micromol, kg: kilogram, min: minute; n: number of patients; %: percentage.

Figure 2 Endogenous glucose production while on PI and after PI-withdrawal

μmol: micromol, kg: kilogram, min: minute. The line represents the mean.
Peripheral glucose metabolism (Figure 3, Table 3)

During fasting at study entry the glucose oxidation expressed as percentage of total glucose disposal was 30.5 ± 10.4 %. The glucose oxidation expressed as percentage of total glucose disposal increased by 18.4 (4.7 –32) % to 48.8 ± 15.6 % after PI withdrawal. At week 12 no differences were observed in the changes in glucose oxidation during fasting between the participants randomized to immediate or deferred withdrawal of PI. (p = 0.88)

At study entry the hyperinsulinemia during the clamp increased total glucose disposal by 32 (17 –47) % from the fasting value. After PI withdrawal this increase was 21 (4 – 38) %. At study entry total glucose disposal during the clamp was 21.3 ± 4.9 µmol/kg/min. Total glucose disposal during the clamp did not change after PI withdrawal. Of note, glucose oxidation expressed as percentage of total glucose disposal was 36.8 ± 12.7 % at study entry and did increase significantly by 11.6 (1.5–21.7) % to 48.4 ± 8.7 % after PI withdrawal.

At week 12 no differences were observed in either total glucose disposal or glucose oxidation both during the clamp between the participants randomized to immediate or deferred withdrawal of PI. (p = 0.26 and p= 0.62 , for total glucose disposal and glucose oxidation, respectively)

Glycerol turnover (Figure 4, Table 3)

Fasting glycerol turnover was 2.6 ± 0.6 µmol/kg/min at study entry. PI withdrawal resulted in a decrease in fasting glycerol turnover of 0.8 (-1.4 − -0.3) µmol/kg/min to 1.8 ± 0.3 µmol/kg/min. During the clamp, glycerol turnover was 1.8 ± 0.6 µmol/kg/min which decreased by 0.6 (-1.2 − -0.1) µmol/kg/min to 1.2 ± 0.5 µmol/kg/min after PI-withdrawal. At study entry the clamp decreased glycerol turnover by 32 (40 – 23) %, after PI withdrawal by 35 (54 – 16) %.
Both during the clamp and under fasting conditions at week 12 no differences were observed in the changes in glycerol turnover between the participants randomized to immediate withdraw the protease inhibitor and deferred. (p = 0.5 and p = 1.0 during the clamp and fasting, respectively)

**Figure 3** Total glucose disposal and glucose oxidation while on PI and after PI withdrawal

**Figure 4** Glycerol turnover while on PI and after PI withdrawal

\[ \mu \text{mol: micromol, kg: kilogram, min: minute. The lines represent the mean.} \]
Discussion

The results of our study demonstrate that two years of withdrawal of PI in HIV-1 infected patients with severe lipodystrophy does not result in an improvement of disturbed fat distribution, while glucose metabolism and lipolysis are partially restored and markedly diminished, respectively.

Although fasting glucose production decreased after PI withdrawal it remained elevated when compared to values previously obtained both in healthy volunteers and in untreated HIV-infected patients. [12,28] However, despite two years of PI withdrawal insulin-stimulated peripheral glucose disposal did not improve. Nonetheless, we did observe significant improvements in intracellular glucose metabolism reflected by a significant increase in glucose oxidation, both under fasting conditions and during insulin infusion. Likewise, the rate of lipolysis which was increased during fasting in HIV-1 infected patients with lipodystrophy, returned towards the levels we have previously measured in healthy volunteers. [13] All of these metabolic changes occurred in the absence of significant improvements in body composition.

The mechanism by which PI acutely cause peripheral insulin resistance involves a direct inhibition of GLUT-4. [15-17,29] Interestingly, in the severely lipodystrophic HIV-1 infected patients enrolled into our study, the extended withdrawal of PI did not result in significant improvement of insulin-stimulated peripheral glucose disposal. This may indicate that long-term treatment with PI irreversibly impairs the intrinsic activity of GLUT-4. Alternatively, this may be explained by direct or indirect changes in glucose metabolism induced by persisting lipodystrophy. However, both fasting and insulin-stimulated intracellular glucose oxidation did improve. Given that glucose oxidation is inhibited by free fatty acids and stimulated by insulin the marked decrease in lipolysis seen after PI withdrawal may very well be
effects of PI discontinuation on glucose and lipid metabolism

responsible for the improvement in glucose oxidation. Taken together, our data supports the notion proposed by Behrens et al,[11] that mechanisms, in addition to inhibition of GLUT-4 activity, are responsible for the changes in glucose metabolism seen in HIV-infected patients with established lipodystrophy.

The trend for lipolysis to normalize following PI withdrawal occurred independent of any changes in concentrations of plasma insulin, a potent inhibitor of lipolysis, epinephrine and cortisol, both potent stimulators of lipolysis, and fat distribution. An explanation for the decrease in lipolysis might be a lowered tonus of the sympathetic nervous system reflected by a decrease in norepinephrine concentrations. Although the decrease of -0.7 (-1.5 - 0.02) nmol/l in norepinephrine after PI withdrawal was not statistically significant in the majority of patients plasma norepinephrine concentrations decreased after withdrawal of PI. In HIV-1 infected lipodystrophic patients using PI norepinephrine concentrations are elevated compared to untreated HIV-1 infected individuals [12,30], suggestive of an overall increased tonus of the sympathetic nervous system. Lipolysis nevertheless is similarly increased both in HIV-1 infected patients with lipodystrophy and in treatment-naïve HIV-1 patients when compared to in healthy volunteers. [12,13] This indicates that although the factor responsible for stimulating lipolysis in uncontrolled HIV infection has disappeared, it must have been replaced by another mechanism. Based on our findings we propose that PI in as yet unexplained fashion induce changes in the tonus of the sympathetic nervous system resulting in increased whole body lipolysis that gradually normalises after PI withdrawal.

A potential bias of our study might be the fact that patients aged during the two years of follow up which thereby may have counteracted any improvement PI withdrawal might have had on insulin sensitivity. Although aging is associated with a decrease in total glucose disposal, this decrease was demonstrated to be as low as 0.9 μmol/kg/min per
decade of life.[31] Furthermore, it seems that the age-related decrease in insulin sensitivity is mainly due to a reduction in insulin stimulated glucose oxidation.[32] Of note, glucose oxidation significantly improved. Moreover, glucose metabolism was also assessed at 12 and 36 weeks after PI withdrawal, and no acute improvements in insulin sensitivity were observed. (data not shown) Taken together this makes it highly unlikely that aging explains why total glucose disposal did not improve significantly.

Another limitation of our study is the small sample size and the fact that one patient died before completing 96 weeks and that in two out of the eight patients PI had to be re-introduced because of virological failure. This occurred in patient 5 at week 36 and in patient 1 at week 7. In both patients virological failure likely was the result of multiple pre-existing zidovudine resistance conferring mutations which were retrospectively demonstrated in stored specimens (data not shown). Unfortunately, at the time the study was designed and these patients were enrolled the results of trials showing such patients to be at increased risk of virological failure when replacing PI by abacavir were not yet available.[33] The small sample size of our study most likely explains why moderate improvements in fat distribution, which were reported following PI withdrawal in larger controlled trial[19,20], were not observed. Nevertheless, in spite of a limited sample size we were able to demonstrate significant changes in glucose metabolism and lipolysis.

In summary, PI replacement by abacavir in severe lipodystrophic HIV-1 infected patients after 96 weeks resulted in a clear trend for lipolysis to normalize and in a significant improvement in glucose oxidation. In contrast, fasting endogenous glucose production improved modestly, while insulin stimulated glucose disposal and fat distribution did not change significantly. Taken together, this suggests that mechanisms in addition to inhibition of GLUT-4 activity are responsible for some of the changes in glucose metabolism seen in HIV-infected patients with
established lipodystrophy. Finally, we propose that disappearance of PI-mediated increases in sympathetic nervous system activity may underlie the decrease in lipolysis following PI withdrawal.
Chapter 8

References


