Adipocyte-derived hormone levels in HIV lipodystrophy

Lisa Kosmiski1*, Daniel Kuritzkes2, Kenneth Lichtenstein3 and Robert Eckel1

1Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Colorado Health Sciences Center, Denver, Col., USA
2Department of Medicine, Division of Infectious Disease, University of Colorado Health Sciences Center, Denver, Col., USA
3Department of Medicine, Rose Medical Center, Denver, Col., USA

*Corresponding author: Tel: +1 303 315 8443; E-mail: Lisa.Kosmiski@uchsc.edu

Objective: Despite evidence for the role of adipocyte-derived hormones in insulin resistance, little is known about their levels in human lipodystrophic states. We examined the relationships of plasma leptin and adiponectin levels to fat distribution and insulin sensitivity in the HIV lipodystrophy syndrome.

Design: Cross-sectional study

Setting: HIV primary care practices

Patients: HIV-infected men with (n=13) and without (12) lipodystrophy and healthy uninfected controls (12).

Main outcome measures: Plasma adiponectin and leptin levels were measured in the fasting state. Body composition was assessed by physical examination, dual-energy x-ray absorptiometry and computed tomography. Insulin sensitivity (S) was measured using the insulin-modified frequently sampled intravenous glucose tolerance test.

Results: Leptin levels were significantly higher in HIV-infected men with lipodystrophy as compared to HIV-infected controls (5.2 vs 3.0 ng/ml, P=0.01). Across the entire study population, leptin levels were positively correlated with measures of general adiposity. In the HIV-infected patients, leptin levels were negatively correlated with S, after adjustment for fat mass (r=-0.38, P=0.07).

Adiponectin levels were significantly lower in HIV-infected men with lipodystrophy as compared to both HIV-infected and healthy controls (1.6 vs 3.4 µg/ml, P<0.05 and 1.6 vs 6.7 µg/ml, P<0.001, respectively).

Conclusions: Plasma leptin and adiponectin levels are altered in the HIV lipodystrophy syndrome. Adiponectin deficiency may play a role in the insulin resistance associated with HIV lipodystrophy.

Introduction

The HIV lipodystrophy syndrome is characterized by changes in body fat distribution and metabolic disturbances [1]. The metabolic disturbances associated with HIV lipodystrophy include insulin resistance, elevated low-density lipoprotein (LDL)-C and triglyceride levels, and decreased high-density lipoprotein (HDL)-C levels [2]. Changes in fat distribution include regional fat wasting and accumulation. Fat accumulation occurs in the visceral depot and less commonly in the dorsocervical fat pad and female breast. By contrast, loss of fat occurs in the subcutaneous depots of the extremities and face. Manifestations of both regional fat accumulation and fat wasting are present in many patients [3].

Adipose tissue acts not only as a storage depot for triglycerides, but also as an endocrine organ releasing hormones that affect whole-body metabolism such as TNF-α, leptin and adiponectin [4]. In the general population, leptin levels are strongly and positively correlated with fat mass [5]. Adiponectin levels, on the other hand, are strongly and negatively correlated with fat mass [6]. However, adiponectin levels are also low in a mouse model of lipoatrophy [7], suggesting that adiponectin and body fat mass may have a more complicated relationship with low levels in both obesity and lipoatrophy.

The lipodystrophy syndromes in the uninfected general population share a similar metabolic profile with the HIV lipodystrophy syndrome, including severe insulin resistance [8]. However, despite recent evidence for the role of adipocyte-derived hormones in insulin resistance [4], little is known about their levels in human lipodystrophic states. In mice with generalized lipoatrophy, leptin levels are low and leptin...
administration markedly improves insulin resistance independently of its effect on food intake [9]. In a different mouse model of generalized lipoatrophy, adiponectin levels are low and the associated insulin resistance is partially reversed by physiological doses of adiponectin [7]. In the human variant of generalized lipoatrophy, leptin levels are also low and leptin administration has been found to markedly improve insulin sensitivity, although this may be the result of decreased caloric intake [10]. In a recent study of adiponectin levels in human variants of lipodystrophy, adiponectin levels were found to be extremely low in patients with congenital and acquired forms of generalized lipoatrophy [11]. Patients with partial lipodystrophy had higher adiponectin levels as compared to those with generalized lipoatrophy, but subjects with HIV lipodystrophy and healthy controls were not included in this study.

To investigate whether insulin resistance and body fat distribution in HIV lipodystrophy are related to levels of adipocyte-derived hormones, we measured leptin and adiponectin levels in HIV-infected men with and without lipodystrophy and in healthy uninfected controls.

**Research design and methods**

HIV-infected men were recruited from local clinics. Subjects gave written informed consent under a protocol approved by the Institutional Review Board at the University of Colorado Health Sciences Center. Healthy HIV-uninfected controls matched for gender, age and body mass index were obtained from a body composition database. Subjects were included in the group with lipodystrophy (HIV-LD) if the subject, his primary care provider and the principal investigator reported accumulation of central fat in addition to loss of fat from at least one depot during the course of protease inhibitor (PI) therapy, and the waist/hip ratio (WHR) was >0.95. This ratio was chosen as an objective indicator of central fat deposition because it is widely accepted as an indicator of central adiposity in the general population. All of the HIV-infected subjects were treated with PIs for at least 1 year and all were on a PI at the time of the study. In addition, all of these patients were on at least one nucleoside reverse transcriptase inhibitor (NRTI). PI-treated subjects were included in the group without lipodystrophy (HIV-infected controls) if the subject, his primary care provider and the principal investigator agreed that the patient showed no signs of lipohypertrophy or lipoatrophy, and the WHR ratio was <0.95. None of the patients were receiving lipid-lowering therapy or medications to treat insulin resistance or diabetes. High-dose glucocorticoid therapy in the past year, an active infection or malignancy, and a fasting glucose ≥110 mg/dl were exclusion criteria. All subjects were studied during admissions to the General Clinical Research Center.

The insulin-modified frequently sampled IV glucose tolerance test (FSIGTT) was used to assess insulin sensitivity in the HIV-infected subjects [12]. Prior to the FSIGTT, subjects consumed a eucaloric diet providing 30% of calories as fat, 15% as protein and 55% as carbohydrate for 3 days. Subjects were asked not to exercise in the 48 h prior to the insulin sensitivity test. In the HIV-infected patients, blood samples for analysis of leptin, adiponectin, insulin, glucose, CD4 counts and viral load measurements were collected after a 12 h overnight fast. In the healthy controls, stored serum was used for analysis of leptin and adiponectin levels. Plasma leptin was measured by ELISA and adiponectin by RIA (Linco Research, Inc). The limit of detection for leptin and adiponectin is 0.05 and 2.0 ng/ml, respectively. For leptin, the inter- and intra-assay coefficients of variation were ≤6.2 and ≤7%, respectively. For adiponectin, the inter- and intra-assay coefficients of variation were both <7%. Glucose was measured by a glucose hexokinase assay and insulin by competitive radioimmunoassay (Pharmacia).

In the HIV-infected subjects, waist circumference was measured at the level of the umbilicus with the subject standing and after a normal expiration. Hip circumference was measured at the level of the greatest gluteal protuberance. All measurements were done by the same investigator. Body composition was determined by dual-energy x-ray absorptiometry using a model DPX-1Q whole body scanner (Lunar Radiation Corp., Madison, Wis., USA), and visceral adipose tissue (VAT) area and abdominal subcutaneous adipose tissue (SAT) area were estimated by abdominal CT performed on a GE9800 scanner as previously described [13]. Healthy controls were studied on the same DEXA scanner.

Group means were compared by one-way analysis of variance or by t-test where appropriate. When data was not normally distributed, median values were compared by Kruskal-Wallis one-way analysis of variance on ranks. Pearson's product-moment correlation coefficients were used to quantify the associations between variables. Stepwise forward regression analysis was used to measure the contribution of variables to the value of independent variables in a multiple linear regression. All statistical analyses were performed with SigmaStat (Version 2.03, SPSS, Chicago, Ill., USA) statistical software.

**Results**

Table 1 shows the characteristics of the men enrolled in the study. Age and body mass index (BMI) were similar between the groups. By design, median WHR
of HIV-LD subjects was significantly greater as compared to the HIV-infected controls (P < 0.001). Mean CD4 cell counts and median plasma HIV-1 RNA level, duration of HIV disease and duration of PI therapy were not significantly different in the HIV-LD group as compared to the HIV-infected controls. There was no significant difference in DEXA-determined lean body mass (LBM), % body fat or total body fat between the three groups (Table 2). The percent of total body fat located in the trunk as determined by DEXA was significantly greater in the HIV-LD group as compared to both HIV-infected and healthy controls (P = 0.01 and < 0.001, respectively). By contrast, the percent of total body fat present in the extremities was significantly lower in the HIV-LD group as compared to both HIV-infected and healthy controls (P < 0.05 and < 0.001, respectively). The percent of total fat in the trunk was also significantly greater in the HIV-infected controls as compared to the healthy controls (P < 0.01), while the percent of total fat in the extremities was significantly lower in the HIV-infected controls as compared to healthy controls. (P < 0.001). VAT was significantly greater in HIV-LD subjects as compared to HIV-infected controls (P < 0.01), but SAT was not significantly different between these groups. The absolute amount of fat in the trunk as measured by DEXA was not significantly different between the groups despite the significantly greater amount of VAT in the HIV-LD group. The absolute amount of fat in the extremities was, however, significantly lower in both HIV-infected groups as compared to the healthy controls (P < 0.05).

Metabolic parameters are presented in Table 3. Leptin levels were significantly greater in the HIV-LD group as compared to the HIV-infected controls (P < 0.05) but not as compared to healthy controls. However, when adjusted for kg of fat mass, median leptin levels were significantly greater in the HIV-LD groups as compared to both control groups (P < 0.05, data not shown). Adiponectin levels were significantly lower in the HIV-LD group as compared to both HIV-infected and healthy controls (P < 0.05 and < 0.001, respectively). In addition, HIV-infected controls had significantly lower adiponectin levels as compared to healthy controls (P < 0.001). After adjustment for kg of fat mass, median adiponectin levels remained significantly different in the HIV-LD group as compared to both control groups (P < 0.05, data not shown) and the differences were of similar magnitude. Insulin sensitivity (S) was markedly impaired in the HIV-LD subjects and mean S was significantly lower in this group as compared to the HIV-infected controls (P < 0.001). Mean fasting insulin and median triglyceride level were significantly higher in the HIV-LD group as compared to HIV-infected controls (P ≤ 0.001 and ≤ 0.05, respectively).

Across the entire study population, including the healthy controls, leptin levels were significantly and positively correlated with kg of fat mass (r = 0.57, P < 0.001) and with % body fat (r = 0.48, P < 0.01, Table 4). When each of the three groups was analysed separately, leptin and fat mass remained significantly and positively correlated (P < 0.05, data not shown). When leptin levels were adjusted for fat mass, leptin levels did not correlate with percent of total body fat located in the trunk or extremities. Adiponectin levels were not significantly correlated with percent body fat or fat mass. However, when the groups were analysed separately, adiponectin levels were significantly and positively correlated with fat mass in the HIV-LD patients (r = 0.55, P ≤ 0.05) but were significantly and negatively correlated with fat mass in the HIV-infected controls (r = 0.65, P < 0.05). In the healthy controls, adiponectin and fat mass were not significantly correlated. Across the entire study population, adiponectin levels adjusted for kg of fat mass were significantly and negatively correlated with percent of body fat in the trunk (r = 0.57, P < 0.001) but positively correlated with percent of body fat in the extremities (r = 0.54, P < 0.001). In the HIV-infected patients, leptin levels were significantly correlated with fasting insulin levels (r = 0.47, P < 0.05), and were significantly and negatively correlated with $S_i$ (r = 0.52, P < 0.01). However, after

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>HIV lipodystrophy</th>
<th>HIV-infected controls</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.8 ±5.5</td>
<td>37.0 ±4.8</td>
<td>36.3 ±9.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ±3.6</td>
<td>23.2 ±2.8</td>
<td>23.8 ±2.4</td>
</tr>
<tr>
<td>WHR</td>
<td>1.0 (0.99, 1.05)</td>
<td>0.88 (0.87, 0.91)*</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of HIV (years)</td>
<td>12.0 (11, 13)</td>
<td>8.5 (4.5, 13)</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of PI therapy (months)</td>
<td>29.5 (25, 30)</td>
<td>24.0 (20.5, 28.75)</td>
<td>N/A</td>
</tr>
<tr>
<td>HIV-1 RNA levels</td>
<td>&lt;200 (199, 1044)</td>
<td>&lt;200 (199, 199.75)</td>
<td>N/A</td>
</tr>
<tr>
<td>CD4 cell count (10⁹/l)</td>
<td>511 ±296</td>
<td>592 ±275</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Data are means ±SD except WHR, HIV-1 RNA levels, duration of HIV disease and duration of PI therapy, which are reported as median values with 25th and 75th percentiles in parentheses. *P < 0.001.
adjustment for kg of fat mass, the strength of this correlation decreased ($r=–0.38$, $P =0.07$). In a forward stepwise regression model of leptin where the possible determinants were age, fat mass, % of body fat in the trunk, % of body fat in the extremities, SI and fasting insulin levels, fat mass and fasting insulin were independent determinants of plasma leptin concentrations in the HIV-infected patients.

Adiponectin levels were positively correlated with SI ($r=0.69$, $P <0.001$) in the HIV-infected patients; the strength of this correlation increased slightly after adjusting adiponectin for kg of fat mass ($r=0.75$, $P <0.001$). When subjects in the HIV-LD group were analysed separately, adiponectin levels remained strongly and positively correlated with SI ($r=0.65$, $P <0.05$). In a forward stepwise regression model of adiponectin where the possible predictors were age, fat mass, % of body fat in the trunk, % of body fat in the extremities, fasting insulin and SI, % of body fat in the trunk and SI were independent predictors of plasma adiponectin concentrations in the HIV-infected patients.

In a regression model of SI, where the possible determinants of SI in HIV-infected patients were age, fat mass, % trunk fat, % extremity fat and VAT, only fat mass and % trunk fat were independent determinants of SI. However, when adiponectin and leptin were added to the model, only age and adiponectin levels remained as independent determinants of insulin sensitivity in the HIV-infected patients, and measures of body composition and fat distribution were eliminated from the model.

**Discussion**

In summary, we found that leptin levels are higher and adiponectin levels are lower in patients with the HIV lipodystrophy syndrome manifested by central fat accumulation and peripheral fat loss, as compared to both HIV-infected and healthy controls. Leptin levels were positively correlated with general adiposity in both the combined patient population and within each group, but were not correlated with measures of body fat distribution. In contrast, adiponectin levels were significantly correlated with measures of fat distribution but not with general levels of adiposity when all subjects were combined. In HIV-infected patients with lipodystrophy, adiponectin was positively correlated with fat mass, but in HIV-infected controls, adiponectin and fat mass were negatively correlated. Plasma adiponectin levels were also strongly correlated with and were independent predictors of insulin sensitivity in HIV-infected patients. When the HIV-LD group was analysed separately, adiponectin levels remained strongly and positively correlated with

---

**Table 2. Body composition**

<table>
<thead>
<tr>
<th></th>
<th>HIV lipodystrophy</th>
<th>HIV-infected controls</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBM (kg)</td>
<td>57.9 ±6.0</td>
<td>58.6 ±8.2</td>
<td>60.1 ±6.1</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>13.6 ±5.7</td>
<td>12.4 ±2.9</td>
<td>15.4 ±5.4</td>
</tr>
<tr>
<td>% body fat</td>
<td>18.2 ±5.4</td>
<td>17.2 ±2.6</td>
<td>19.6 ±5.4</td>
</tr>
<tr>
<td>% of body fat in the trunk</td>
<td>71.3 ±6.6</td>
<td>63.3 ±7.0 †</td>
<td>53.3 ±5.5 ‰</td>
</tr>
<tr>
<td>kg of fat in the trunk</td>
<td>9.7 ±3.3</td>
<td>7.8 ±2.2</td>
<td>8.2 ±3.0</td>
</tr>
<tr>
<td>% of body fat in the extremities</td>
<td>23.0 ±6.8</td>
<td>29.9 ±6.8*</td>
<td>41.5 ±5.5 †</td>
</tr>
<tr>
<td>kg of fat in the extremities</td>
<td>3.4 ±2.5</td>
<td>3.7 ±1.1</td>
<td>6.4 ±2.4 ‡</td>
</tr>
<tr>
<td>SAT (cm²)</td>
<td>108.8 ±66.5</td>
<td>121.4 ±35.2</td>
<td>Not done</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>195.9 ±71.1</td>
<td>120.7 ±45.5 †</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Data are means ±SD. HIV-LD versus HIV-controls: *$P<0.05$, † $P<0.01$.
HIV-LD versus healthy controls: † $P<0.05$, ‡ $P<0.01$.
HIV-controls versus healthy controls: § $P<0.05$, ¶ $P<0.01$.

---

**Table 3. Insulin sensitivity, triglycerides and adipocyte-derived hormone levels**

<table>
<thead>
<tr>
<th></th>
<th>HIV lipodystrophy</th>
<th>HIV-infected controls</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>5.2 ±2.1</td>
<td>3.1 ±1.7*</td>
<td>3.7 ±1.6</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>1.6 ±0.7</td>
<td>3.4 ±1.7*</td>
<td>6.7 ±1.9 ‡</td>
</tr>
<tr>
<td>SI(22) x10⁻⁶ (min⁻¹/µU/ml)</td>
<td>1.0 ±0.7</td>
<td>3.2 ±1.5 †</td>
<td>Not done</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>22.1 ±10.3</td>
<td>9.5 ±4.4 †</td>
<td>Not done</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>431 (403, 596)</td>
<td>242 (12, 430)*</td>
<td>Not done</td>
</tr>
</tbody>
</table>

Data are means ±SD except for triglycerides, which are reported as a median value with 25th and 75th percentiles in parentheses.
HIV-LD versus HIV controls: * $P<0.05$, † $P<0.001$.
HIV-LD versus healthy controls: † $P<0.001$.
HIV-controls versus healthy controls: § $P<0.001$. 

---

©2003 International Medical Press
levels were normal in two children with familial partial lipodystrophy, leptin levels were significantly lower in affected adults as compared to healthy uninfected controls [17]. However, leptin levels were not adjusted for fat mass. In another study, leptin levels were lower in men with HIV lipodystrophy manifested primarily by fat loss as compared to therapy-naive and healthy controls [20]. Again, leptin levels were not adjusted for fat mass, which is the primary determinant of plasma leptin levels.

In this study, the observed increase in leptin levels in subjects with lipodystrophy may be related to the hyperinsulinaemia that accompanies insulin resistance. Other studies have shown that insulin resistance and insulin levels are correlated with leptin levels independent of fat mass [21,22]. Furthermore, insulin increases leptin synthesis and secretion in adipocytes [23]. There is now convincing evidence that leptin is an anti-steatotic hormone [24]. A accumulation of triglyceride in skeletal muscle is likely a major cause of insulin resistance in this tissue [25,26]. Increases in leptin in the setting of insulin resistance and hyperinsulinaemia may, therefore, be an adaptive response to lessen peripheral steatosis in conditions such as obesity. Leptin levels may also depend on the HIV lipodystrophy phenotype under study. The patients in this study had lipodystrophy manifested by both central fat accumulation and peripheral fat loss and this may explain their higher leptin levels.

Adiponectin deficiency may play a major role in the insulin resistance of the lipodystrophy syndrome. This study also found that HIV-infected men with no clinical evidence of lipodystrophy had altered body fat distribution and lower adiponectin levels as compared to healthy controls. The HIV-infected controls had significantly lower amounts of subcutaneous fat in the extremities as compared to healthy controls. Perhaps adiponectin is lower in these patients because subcutaneous adipose tissue is an important site of production for this hormone. However, we know of no published data regarding the relationship of adiponectin to body fat distribution. Alternatively, adiponectin levels may be low in HIV-infected patients without clinical evidence of HIV lipodystrophy secondary to HIV infection itself or to antiretroviral therapy.

In the general population, the relationship of leptin levels to body fat distribution is also unclear. Leptin expression per gram of adipose tissue is greater in subcutaneous as compared to visceral adipose tissue [14] and in some studies, the strongest predictor of plasma leptin levels is the amount of subcutaneous adipose tissue [15,16].

There is little information regarding plasma leptin concentrations in patients with partial forms of lipodystrophy. In an extended Canadian kindred with familial partial lipodystrophy, leptin levels were significantly lower in affected adults as compared to unaffected family controls [17]. However, leptin levels were not adjusted for fat mass. In another study, leptin levels were normal in two children with familial partial lipodystrophy [18]. In the HIV lipodystrophy syndrome, one study found that leptin levels were higher in both HIV-infected subjects with and without lipodystrophy as compared to healthy uninfected controls but were similar in the two HIV-infected groups [19]. However, leptin levels were not adjusted for fat mass. In another study, leptin levels were lower in men with HIV lipodystrophy manifested primarily by fat loss as compared to therapy-naive and healthy controls [20]. Again, leptin levels were not adjusted for fat mass, which is the primary determinant of plasma leptin levels.

Like leptin, adiponectin appears to be an anti-steatotic hormone. In both lipoatrophic and obese mice, adiponectin administration decreases insulin resistance by decreasing triglyceride content in both liver and muscle [7]. The decrease in peripheral triglycerides was due to an increase in expression of molecules involved in fatty-acid transport, combustion and energy dissipation. Adiponectin levels are also lower in obese patients with Type 2 diabetes as compared to obese controls [27], suggesting a link between adiponectin and insulin sensitivity in humans. Although the physiological role of adiponectin in human metabolism remains unclear, it is possible that the insulin resistance of HIV lipodystrophy is related, in part, to a relative deficiency of adiponectin. Further

### Table 4. Correlation coefficients between hormone levels and body composition measurements across the entire study population, and between hormone levels and insulin sensitivity in HIV-infected patients

<table>
<thead>
<tr>
<th></th>
<th>Leptin</th>
<th>Adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>% body fat</td>
<td>0.48†</td>
<td>0.11</td>
</tr>
<tr>
<td>Fat mass</td>
<td>0.57‡</td>
<td>0.09</td>
</tr>
<tr>
<td>% body fat in trunk</td>
<td>0.32</td>
<td>-0.57‡</td>
</tr>
<tr>
<td>% body fat in extremities</td>
<td>-0.26</td>
<td>0.54‡</td>
</tr>
<tr>
<td>SI</td>
<td>-0.38</td>
<td>0.75‡</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>0.47*</td>
<td>0.47*</td>
</tr>
</tbody>
</table>

Leptin and adiponectin were adjusted for fat mass when correlated with % body fat in trunk and extremities, SI, and fasting insulin. Data presented are Pearson Product correlation coefficients. *P<0.05, † P<0.01, ‡ P<0.001.
studies are warranted to confirm these findings and to determine if reductions in plasma adiponectin levels are associated with use of specific antiretroviral drugs or body fat changes, or both. Other, as yet undetermined factors, may also explain the relative adiponectin deficiency associated with the HIV infection.

Acknowledgements

The authors thank the patients who participated in this study, the nurses and the personnel of the CORE laboratory of the General Clinical Research Center. Supported in part by grants from the NIH (M01RR00051, K23 RR16069, K24 RR16482).

References


