Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy

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Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. Am J Physiol Endocrinol Metab 286: E261–E271, 2004. First published October 7, 2003; 10.1152/ajpendo.00056.2003.—The lipodystrophy syndrome (adipose tissue redistribution and metabolic abnormalities) observed with highly active antiretroviral therapy (HAART) during human immunodeficiency virus (HIV) infection may be related to increased proinflammatory cytokine activity. We measured acute cytokine (TNF-α, IL-6, leptin), glycerol, and lactate secretion from abdominal subcutaneous adipose tissue (SAT), and systemic cytokine levels, in HIV-infected subjects with and without lipodystrophy (HIVL+ and HIVL−, respectively) and healthy non-HIV controls. Lipodystrophy was confirmed and characterized as adipose tissue redistribution in HIVL+ compared with HIVL− and controls, by dual-energy X-ray absorptiometry and by whole body MRI. TNF-α secretion from abdominal SAT and circulating levels of IL-6, soluble TNF receptors I and II, and insulin were elevated in HIVL+ relative to HIVL− and/or controls, particularly in HIVL+ undergoing HAART. In the HIV-infected group as a whole, IL-6 secretion from abdominal SAT and serum IL-6 were positively associated with visceral fat and were negatively associated with the relative amount of lower limb adipose tissue (P < 0.01). Decreased leptin and increased lactate secretion from abdominal SAT were specifically associated with HAART. In conclusion, increased cytokine secretion from adipose tissue and increased systemic proinflammatory cytokine activity may play a significant role in the adipose tissue remodeling and/or the metabolic abnormalities associated with the HIV-lipodystrophy syndrome in patients undergoing HAART.

The etiology of these adipose tissue changes and of the associated metabolic abnormalities is unclear but is likely to be multifactorial. Several studies have found associations between the development of fat redistribution and antiretroviral drugs, with distinct and interacting influences of protease inhibitors (PI) and nucleoside reverse transcriptase inhibitors (NRTI) (9, 45, 66). In vitro studies have confirmed direct effects of both PI and NRTI on adipose tissue metabolism. PI have been reported to induce insulin resistance, to decrease glucose transport and adipogenesis (8, 48, 49, 57, 60, 70), to either increase (51) or decrease preadipocyte differentiation in 3T3-L1 adipocyte cell lines (8, 14), and to increase apoptosis in human subcutaneous adipose tissue (13). In addition, NRTI have been shown to have a toxic effect on mitochondrial DNA in human subcutaneous adipose tissue (45, 63, 67).

Alternative hypotheses of pathogenesis of the adipose tissue changes, aside from possible direct effects of antiretroviral agents, have been given less attention. However, it has been shown that drug exposure alone is not always sufficient to produce changes in adipose tissue morphology and metabolism (27, 38, 41, 52).

Several investigators have proposed a relationship between elevated inflammatory activity and adipose tissue changes in HIV infection (3, 23, 40, 48, 50). Elevated serum concentrations of soluble TNF-α receptor II have been shown to be related to lipodystrophy and insulin resistance in HIV-infected patients (50). TNF-α, IL-6, and other cytokines are produced in many cell types, including adipocytes, and are associated with elevated lipolysis and insulin resistance in non-HIV individuals (30, 31, 36, 37). Furthermore, elevated local production of TNF-α in the adipose tissue may downregulate several genes involved in adipogenesis and fat cell differentiation (20, 21, 64), potentially leading to adipocyte apoptosis and lipolatrophy (3, 56).

Recently, it was shown that HIV-infected patients with lipodystrophy have increased expression of TNF-α in abdominal subcutaneous adipose tissue and decreased expression of leptin and adipocyte differentiation markers relative to healthy, non-HIV controls (3). However, the secretion of TNF-α and other adipose tissue-derived inflammatory markers and their potential relationships to in vivo adipose tissue redistribution in HIV infection have not been previously examined.

In the present study, we measured short-term release of cytokines, glycerol, and lactate from abdominal subcutaneous
Adipose tissue in vitro, as well as circulating levels of soluble TNF-α receptors, IL-6, leptin, and lactate in HIV-infected subjects with and without lipodystrophy (HIVL+ and HIVL−, respectively, as determined by self-report of body shape change) and in healthy control subjects. We have also related these to measurements of regional fat distribution determined by dual-energy X-ray absorptiometry (DEXA) and by whole body magnetic resonance imaging (MRI).

**METHODS**

**Subjects.** This was a cross-sectional study that included 27 HIVL+, 16 HIVL−, and 12 healthy controls. Group assignment was made by visual assessment of a medical doctor familiar with the body fat changes that occur with lipodystrophy and by patients’ self-report of body shape changes. Pertinent inclusion criteria were the following: age >18 yr, ability to give consent, stable health, being nonpregnant (where applicable), without any active malignancy, and not on any investigational agents. The studies were approved by the Institutional Review Board of St. Luke’s-Roosevelt Institute for Health Sciences, and all subjects gave written informed consent.

**Body composition.** Body weight was measured to the nearest 0.1 kg (Weight Tronix) and height to the nearest 0.5 cm with a stadiometer (Holtain, Crosswell, UK). Waist and hip circumferences were measured as described (1), and the waist-to-hip circumference ratio (WHR) was calculated.

Total visceral (VAT) and subcutaneous adipose tissue (SAT) masses were measured using MRI-derived cross-sectional images of the total body, which involves the acquisition of ~40 axial images of 10-mm thickness at 40-mm intervals from head to toe (15, 59). All MRI scans were analyzed by the same observer using image analysis software (sliceOMatic v. 4.0; TomoVision, Montreal, QC, Canada) on a Gateway PC (Gateway, North Sioux City, SD). Volume estimates of adipose tissues were determined from the respective areas (15). The difference in results on repeated image analysis in our laboratory averages 1.1% for adipose tissue (22). The following volumes were calculated: total adipose tissue, VAT, and SAT. SAT distribution was also determined, i.e., SAT volumes in the legs (Leg SAT) and in the trunk (Trunk SAT) as a percentage of total SAT (%Leg SAT and %Trunk SAT, respectively). The legs included all SAT inferior to the greater trochanter, and the trunk included all SAT superior to the level of the trochanter (including abdominal SAT biopsied below the umbilicus for adipose tissue studies; see below).

Total body fat mass and fat-free mass (composed of soft lean tissue and bone mineral) were measured with a whole body DEXA scanner (version 3.6 software, DPX; Lunar Radiation, Madison, WI) as described (29). The difference for repeated measurements in our laboratory for DEXA averages 1.2% for fat-free body mass and 4.7% for fat mass. Percent total body fat and the regional fat distribution (fat in the legs and trunk as %total body fat, %Leg Fat, and %Trunk Fat, respectively) were also calculated.

**Measurements of circulating factors.** Serum concentrations of soluble TNF-α receptor types I and II (sTNF-R1 and sTNF-RII, respectively) and IL-6 were determined by ELISA (R&D Systems, Minneapolis, MN). Serum leptin and insulin levels were measured by RIA (Linco Research, St. Charles, MO). Serum glucose was determined using a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA). The interassay coefficients of variation for assays of serum sTNF-R1, sTNF-RII, IL-6, leptin, insulin, and glucose were 6.1, 4.1, 4.5, 4.5, and <3%, respectively. Serum lactate was measured in eight HIVL+, five HIVL−, and eight control subjects by commercial assay (Quest Diagnostics, Teterboro, NJ).

**Adipose tissue biopsy.** Abdominal SAT and dorsocervical (DC) adipose tissue biopsies were performed in the morning after an overnight fast. The skin was anesthetized (2% lidocaine; Elkins-Sinn, Cherry Hill, NJ), and adipose tissue was obtained using a blunt-ended needle designed for liposuction (3-mm Spiriroti cannula; Unitech Instruments, Fountain Valley, CA). All 55 subjects underwent biopsy of abdominal SAT. Eight HIV+ subjects who had visible buffalo humps, or DC fat pads, also underwent adipose tissue biopsy in this area.

**Determination of adipocyte size.** Adipocytes were isolated by collagenase digestion (34) in 41 of the 55 subjects (19 HIVL+, 12 HIVL−, and 10 controls). Diameters of ≥200 cells from the adipocyte suspension were then measured directly using a microscope with an ocular micrometer. A frequency distribution plot of cell diameters (range 17.5–210 μm, with 17.5-μm intervals) was used to determine the mean adipocyte diameter and standard deviation about the mean; frequency of the mean adipocyte volume and surface area were calculated (34). Mean adipocyte size (weight, μg lipid/cell) was calculated from cell volume, assuming that the density of lipid is equal to that of triolein (0.915 g/fi).

**Short-term adipose tissue release of cytokines and metabolites.** Acute cytokine (TNF-α, IL-6, leptin) and metabolite (glycerol and lactate) secretion from adipose tissue was determined as described (62). Biopsied adipose tissue was minced into 5- to 10-mg fragments, and aliquots of fresh tissue were incubated for 3 hr in Medium 199 (with Earle’s salts and 25 mM HEPES; Gibco Life Technologies, Grand Island, NY) containing 1% BSA (CRG-7; Intergen, Purchase, NY) under a 95% O2 -5% CO2 atmosphere in a shaking water bath (60 cycles/min, 37°C). After incubation, medium was frozen at −70°C for subsequent determination of cytokine and metabolite release.

We were unable to obtain sufficient adipose tissue to measure cytokine and metabolite release in one HIVL− and two HIVL+ subjects.

TNF-α and IL-6 release into incubation medium was determined by ELISA (R&D Systems). For the IL-6 assay, samples were diluted 1:200 with the same buffer used in the incubations to obtain values within the linear range of the assay (3.1–300 pg/ml). The interassay coefficient of variation for the TNF-α assay (culture medium) is 7.6% and for the IL-6 assay 2.7%. Leptin release into adipose tissue incubation medium was measured by RIA (Linco Research). Glycerol and lactate release was analyzed in neutralized perchloric acid extracts of adipose tissue incubation medium (5, 43) by means of a Perkin-Elmer LS50B Luminescence Spectrometer (Perkin-Elmer, Beaconsfield, Buckinghamshire, UK). The interassay coefficient of variation in our laboratory for the leptin assay is 4.5%, for glycerol 7.2%, and for lactate 6.2%.

**Adipose tissue heparin-releasable lipoprotein lipase activity.** Lipoprotein lipase (LPL) activity was determined in abdominal SAT from a subset of subjects (15 HIVL+, 4 HIVL−, control; 28 total). After biopsy, aliquots of finely minced adipose tissue were immediately frozen at −70°C for subsequent determination of heparin-releasable LPL activity, as previously described (19). Units of LPL activity were defined as the activity that catalyzed the release of 1 μmol nonesterified fatty acid (NEFA) per hour. Day-to-day assay variation was corrected by a pooled rat postheparin plasma standard curve.

Additional aliquots of biopsied adipose tissue were immediately frozen at −70°C for later determination of adipose tissue lipid content (17). The number of adipocytes per gram of adipose tissue was then derived by dividing the lipid content per gram of adipose tissue by the mean lipid weight per adipocyte. LPL activity per 106 adipocytes was calculated by dividing the activity per gram of adipose tissue by the adipocyte number per gram of tissue.

**Statistical analysis.** Data in the text and tables are reported as means ± SD, whereas data in the figures are reported as means ± SE. Data that were not normally distributed across all groups, including body composition (SAT, VAT, BMI, Leg SAT, Trunk SAT, Leg Fat, Trunk Fat), circulating factors, and adipose tissue TNF-α, IL-6, and lactate release, were natural log transformed before statistical analysis.

Differences between experimental groups (HIVL+, HIVL−, and control) and between groups with different treatment regimens (NNRTI and nonNNRTI [NNRTI]) with regard to body composition, circulating factors, and adipose tissue metabolism were determined by one-way ANOVA.
Gender-by-group interactions were determined by factorial ANOVA for each of the analyses performed. When no interactions were found, results for both sexes were pooled when differences between groups were reported. Post hoc paired t-tests (least significant difference) were used to compare group means, with statistical significance denoted as \( P < 0.05 \).

Metabolic differences between abdominal SAT and DC adipose tissue depots were compared by paired t-tests.

The general linear model was used to determine homogeneity of slopes within experimental groups (HIVL+, HIVL−, and control) for all analyses of covariance and to determine regression coefficients for the relationships of circulating inflammatory markers or their adipose tissue release with fat distribution variables (VAT, SAT, %Leg Fat, and %Leg SAT) and serum insulin levels. Statistical analyses were performed using STATISTICA for Windows, release 6.0 (StatSoft, Tulsa, OK).

RESULTS

Differences in body composition in HIV+ and HIV− subjects. Subject characteristics (body composition and metabolic variables) in the two HIV groups and in the control group are shown in Table 1. All HIV-infected subjects (HIVL+ and HIVL−) were older (\( P < 0.02 \)) and had lower absolute amounts of total SAT, Leg Fat, and Leg SAT (\( P < 0.05 \)) compared with control subjects. BMI was not significantly different among the three groups.

Compared with control subjects, the HIVL+ subjects had similar percent body fat, VAT, Trunk Fat, and Trunk SAT but had significantly lower absolute and relative amounts of fat in the lower limbs (Leg Fat, Leg SAT, %Leg Fat, and %Leg SAT). Compared with control subjects, the HIVL− patients had lower total body fat, VAT, and overall lower total SAT (including all SAT compartments) but had similar lower limb fat distribution (%Leg Fat and %Leg SAT). Compared with the HIVL− group, the HIVL+ patients had similar age, BMI, and percent body fat but significantly greater truncal adiposity (Trunk Fat, Trunk SAT, and VAT) and significantly lower relative amounts of fat in the lower limbs (%Leg Fat and %Leg SAT).

In summary, HIVL+ subjects had decreased adiposity in the legs compared with controls. HIVL+ subjects also had increased VAT and a redistribution of SAT from the lower to the upper body compared with the HIVL− subjects.

Insulin levels were significantly greater in HIVL+ compared with HIVL− (\( P < 0.04 \)) and control subjects (\( P < 0.03 \),

Table 1. Subject characteristics in HIVL+, HIVL−, and healthy control subjects

<table>
<thead>
<tr>
<th></th>
<th>HIVL+</th>
<th></th>
<th>HIVL−</th>
<th></th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (M/F)</td>
<td>n (M/F)</td>
<td>n (M/F)</td>
<td></td>
<td>[n = 12 (5 M, 7 F)]</td>
</tr>
<tr>
<td>Age, yr</td>
<td>44.6±7.6*</td>
<td>27 (17/10)</td>
<td>41.3±5.9*</td>
<td>16 (11/5)</td>
<td>34.3±9.3†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.8±5.0*</td>
<td>27 (17/10)</td>
<td>26.2±5.5*</td>
<td>15 (10/5)</td>
<td>29.5±5.8*</td>
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<tr>
<td>WHR</td>
<td>0.91±0.2*</td>
<td>22 (13/9)</td>
<td>0.89±0.05*</td>
<td>14 (9/5)</td>
<td>0.89±0.07*</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>20.5±11.6++</td>
<td>21 (12/9)</td>
<td>15.1±12.3*</td>
<td>15 (10/5)</td>
<td>28.3±14.7†</td>
</tr>
<tr>
<td>% Body fat§</td>
<td>26.7±11.0++</td>
<td>22 (13/9)</td>
<td>19.8±12.0*</td>
<td>15 (10/5)</td>
<td>34.8±12.6†</td>
</tr>
<tr>
<td>VAT, liters‡</td>
<td>4.1±2.4*</td>
<td>24 (15/9)</td>
<td>1.9±0.8†</td>
<td>14 (10/4)</td>
<td>3.0±1.3*</td>
</tr>
<tr>
<td>SAT, liters‡</td>
<td>22.4±11.8*</td>
<td>24 (15/9)</td>
<td>17.1±8.2*</td>
<td>14 (10/4)</td>
<td>31.6±15.0†</td>
</tr>
<tr>
<td>Trunk Fat, kg§</td>
<td>12.2±5.4*</td>
<td>21 (12/9)</td>
<td>7.3±5.0†</td>
<td>15 (10/5)</td>
<td>13.8±6.6*</td>
</tr>
<tr>
<td>Leg Fat, kg§</td>
<td>5.4±5.5*</td>
<td>21(12/9)</td>
<td>5.7±5.6*</td>
<td>15 (10/5)</td>
<td>10.6±6.4†</td>
</tr>
<tr>
<td>%Leg Fat*</td>
<td>22.4±10.7*</td>
<td>21 (12/9)</td>
<td>35.0±7.7†</td>
<td>15 (10/5)</td>
<td>36.4±5.6*</td>
</tr>
<tr>
<td>Trunk SAT, liters‡</td>
<td>11.1±5.3*</td>
<td>24 (15/9)</td>
<td>6.2±3.2†</td>
<td>14 (10/4)</td>
<td>14.4±9.4*</td>
</tr>
<tr>
<td>Leg SAT, liters‡</td>
<td>8.1±5.9*</td>
<td>24 (15/9)</td>
<td>8.4±4.4*</td>
<td>14 (10/4)</td>
<td>12.4±3.7†</td>
</tr>
<tr>
<td>%Leg SAT‡</td>
<td>30.0±8.0*</td>
<td>24 (15/9)</td>
<td>44.4±5.0†</td>
<td>14 (10/4)</td>
<td>38.7±9.1†</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>172±100*</td>
<td>27 (17/10)</td>
<td>133±126†</td>
<td>16 (11/5)</td>
<td>106±53†</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.9±1.5*</td>
<td>27 (17/10)</td>
<td>4.5±1.2†</td>
<td>16 (11/5)</td>
<td>4.8±1.1*</td>
</tr>
</tbody>
</table>

Data are means ± SD; \( n \) = no. of subjects (M, male; F, female). HIVL+, human immunodeficiency virus (HIV)-infected subjects with lipodystrophy; HIVL−, HIV-infected subjects without lipodystrophy; BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WHR, waist-to-hip ratio; %Leg Fat, percent leg fat in total body fat measured by dual-energy X-ray absorptiometry (DEXA); % Leg SAT, percent leg fat in total subcutaneous fat measured by whole body magnetic resonance imaging (MRI). Variables not exhibiting a normal distribution were natural log transformed before statistical analysis. Group means with different symbols (*, †) are significantly different (\( P < 0.05 \)); ‡ determined by MRI; § determined by DEXA.

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whereas serum glucose was not significantly different among groups (Table 1).

There were significant differences between the sexes (not shown), consistent in both HIV and control groups. In all groups, females were younger than males (38.1 ± 9.3 vs. 43.6 ± 7.1 yr, \( P < 0.02 \)) with significantly higher percent body fat, BMI, WHR, and total SAT (all \( P < 0.02 \)).

Interactions between group assignment and sex were determined by factorial ANOVA for all body composition variables. No significant interactions between experimental group and sex were found for the variables in Table 1, except for %Leg fat (not shown), consistent in both HIV and control groups. In all subjects, %Leg fat was significantly lower in HIVL− compared with HIVL+ and control males, as was true for the entire group (Table 1); however, %Leg SAT was not significantly different in HIVL+ females compared with control females.

For all other results (circulating inflammatory markers and adipose tissue metabolism variables; see next section), no significant interactions between experimental group and sex were found by factorial ANOVA; therefore, these data were pooled for analysis of differences among HIVL+, HIVL− and control groups.

**Circulating inflammatory markers in HIV+ and HIV− subjects.** Circulating levels of inflammatory markers (IL-6, sTNF-R1, sTNF-RII) as well as leptin and lactate levels in HIV-infected and control subjects are shown in Fig. 1. Serum IL-6 was elevated in HIVL+ relative to both HIVL− and control groups \(( P < 0.01; \) Fig. 1A). Serum sTNF-RII levels were significantly elevated in the HIVL+ compared with the control group \(( P < 0.01; \) Fig. 1A). Serum sTNF-RII levels were modestly elevated in HIVL+ compared with HIVL− \(( P = 0.08 \) and in HIVL− compared with controls \(( P = 0.06; \) Fig. 1A). Conversely, serum sTNF-R1 levels were significantly different among HIV and control groups (Fig. 1A). In all subjects, serum IL-6 was highly correlated with circulating levels of sTNF-R1 \(( r = 0.36, P = 0.009 \) but not with sTNF-R1 \(( r = 0.22, P = 0.11 \), sTNF-R1 and sTNF-RII levels were strongly correlated with each other \(( r = 0.65, P < 0.001 \).

Serum leptin levels were lower in HIVL− compared with both HIVL+ and control groups (Fig. 1B), in parallel with the lower absolute amounts of Trunk Fat and Trunk SAT in the HIVL− group (Table 1). Serum lactate was measured in a subset of subjects \(( n = 8 \) HIVL+, 5 HIVL−, and 8 controls; Fig. 1C). These levels were significantly higher in the HIVL+ subjects relative to controls \(( P < 0.04 \) with intermediate levels in the HIVL− subjects.

Analysis of covariance was used to examine effect of group assignment (HIVL+, HIVL− and control) on circulating levels of inflammatory markers (IL-6, sTNF-R1, and sTNF-R2) after adjustment for indexes of fat distribution (VAT, %Leg Fat, and %Leg SAT). In adjusted post hoc analysis, significant differences between groups were the same as those reported in Fig. 1.

**Abdominal SAT metabolism in HIV+ and HIV− subjects.** Group differences in the metabolic characteristics of abdominal SAT obtained by biopsy are shown in Fig. 2. Mean abdominal SAT adipocyte size was smaller in the HIVL− group than in HIVL+ and control groups \(( P < 0.05; \) Fig. 2A). Abdominal SAT adipocyte size across groups, and within each group, was highly correlated with overall adiposity (BMI, %body fat, fat mass, and SAT) and with truncal adiposity (Trunk Fat and Trunk SAT, both \( P < 0.001 \)) but was not significantly correlated with VAT \(( r = 0.3, P = 0.08 \). Thus differences in abdominal SAT cell size among groups paralleled the lower amounts of Trunk Fat and Trunk SAT in HIVL− subjects (Table 1).

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**Fig. 2.** Abdominal subcutaneous adipocyte size \(( A \) and adipose tissue release of TNF-α and IL-6 \(( B \), leptin \(( C \), and glycerol and lactate \(( D \) in HIVL+ and HIVL− subjects \(( n = 25 \) and 15, respectively) and healthy control subjects \(( n = 12 \). \( E \). lipoprotein lipase (LPL) activity was determined in 15 HIVL+, 4 HIVL−, and 9 Control subjects. LPL activity is expressed as U/10⁶ adipocytes, with 1 U of LPL activity being equal to the release of 1 μmol nonesterified fatty acids/h. Group means with different symbols \(( *, † \) are significantly different \(( P < 0.05 \).
We further explored whether the decreased abdominal adipocyte size in the HIVL− group was primarily due to a gain of small adipocytes (diameter <70 μm) or to an overall decrease in cell size (i.e., a leftward shift in the distribution of cell diameters). We examined the distributions of adipocyte diameters for each group and found no evidence of a bimodal distribution of adipocyte diameter in HIVL− subjects. Adipocyte diameters were normally distributed in all groups, and the distribution of adipocyte diameter in HIVL− subjects. Adipocyte diameters were normally distributed in all groups, and the distribution curve for HIV− was shifted to the left relative to HIVL+ and controls (data not shown). Therefore, adipocytes in the HIVL− subjects were uniformly smaller than in the HIVL+ and control groups, with no preferential increase in small adipocytes.

TNF-α release from abdominal SAT was significantly higher in the HIVL+ group compared with HIVL− (P = 0.01) and control groups (P = 0.04; Fig. 2B). IL-6 release from abdominal SAT did not differ significantly among groups (P = 0.3; Fig. 2B) but was strongly correlated with TNF-α release in all groups (r = 0.48, P < 0.001).

Leptin release from abdominal SAT (expressed per gram of adipose tissue) was similar in all three groups (P = 0.9; Fig. 2C) despite differences in adipocyte size.

Glycerol release from abdominal SAT (expressed per gram of adipose tissue) was greater in the HIVL− than in the control group (P = 0.01), with intermediate values in the HIVL+ group (Fig. 2D). In all three groups combined, glycerol release per gram of adipose tissue was negatively related to adipocyte size (r = −0.32, P < 0.05).

We also calculated glycerol release, expressed per 10^6 adipocytes, in a subset of subjects (n = 15 HIVL+, 3 HIVL−, 9 control) for whom data on adipose tissue lipid content were available (data not shown). In these subjects, glycerol release per 10^6 adipocytes was also elevated in HIVL− relative to controls (828 ± 281 vs. 461 ± 179 nmol·10^−6 cells·3 h−1, P = 0.06), with intermediate values in HIVL+ (630 ± 434 nmol·10^−6 cells·3 h−1).

Lactate release from abdominal SAT was greater in both HIV groups compared with the control group (P < 0.05; Fig. 2D). In all subjects combined, the secretions of glycerol and lactate were strongly related to each other (r = 0.37, P < 0.01). In addition, glycerol release was marginally correlated with IL-6 release (r = 0.25, P = 0.08), and lactate release was marginally correlated with TNF-α release (r = 0.28, P = 0.06).

LPL activity, a marker of adipocyte triglyceride synthesis, was not significantly different among groups (P > 0.7; Fig. 2E), nor were there significant group differences in LPL activity expressed per gram of adipose tissue or per cell surface area (not shown).

Comparison of adipose tissue metabolism in abdominal subcutaneous and DC depots. In a subset of HIVL+ subjects with visible DC fat pads (n = 8), we biopsied both abdominal SAT and DC adipose tissue and compared adipocyte size and cytokine and metabolite release between these two depots (Fig. 3). Adipocyte size was similar between the two depots (Fig. 3A); however, TNF-α and lactate release was significantly greater in abdominal SAT than in DC fat (P = 0.04; Fig. 3, B and D). IL-6 release was higher in abdominal SAT than in DC fat, but this difference was not significant (P = 0.14; Fig. 3B). Leptin and glycerol release values were also elevated in abdominal SAT compared with DC fat, but these failed to reach statistical significance (P < 0.09; Fig. 3, C and D).

Relationships of circulating and adipose tissue proinflammatory cytokines to body fat distribution and serum insulin in HIV-infected subjects. We further examined the relationships between proinflammatory cytokines (circulating levels of inflammatory markers and abdominal SAT cytokine release) and measures of fat distribution in HIV-infected subjects with and without lipodystrophy (Table 2). Slopes of these relationships were not significantly different between HIVL+ and HIVL−; therefore, the pooled correlation coefficients for both HIV groups are shown.

Table 2. Correlation coefficients for relationships of circulating inflammatory markers and abdominal SAT cytokine release with body composition variables in HIV-infected subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Circulating Levels</th>
<th>Adipose Tissue Release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sTNF-RI</td>
<td>sTNF-RII</td>
</tr>
<tr>
<td>VAT, liters*</td>
<td>0.30</td>
<td>0.28</td>
</tr>
<tr>
<td>SAT, liters*</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>%Lg SAT*</td>
<td>−0.31</td>
<td>−0.27</td>
</tr>
<tr>
<td>%Lg Fat*</td>
<td>−0.09</td>
<td>−0.21</td>
</tr>
</tbody>
</table>

For circulating levels of inflammatory markers, n = 43 subjects (27 HIVL+, 16 HIVL−). For adipose tissue cytokine release, n = 40 (25 HIVL+, 15 HIVL−). sTNF-RI and -RII soluble tumor necrosis factor receptors I and II, respectively. Variables not normally distributed were log transformed before statistical analysis. *Determined by whole body MRI; †determined by DEXA. ‡P < 0.05; §P < 0.01.
To determine whether there are interactive or additive effects of HAART status (defined as taking NRTI or PI alone or in any combination) and fat redistribution (HIVL+ and HIVL−) on serum inflammatory markers and adipose tissue metabolic characteristics, we performed a 2 × 2 ANOVA, with lipodystrophy (HIVL+ and HIVL−) and HAART status (HAART+ and HAART−) as factors. Although no statistically significant interactions were found and some of the groups were small in number, we present significant differences from the post hoc analyses (Figs. 4 and 5).

Figure 4 shows circulating levels of inflammatory markers by lipodystrophy and HAART status. Serum sTNF-RI was significantly elevated in HAART+ compared with HAART−, but only in HIVL+ (Fig. 4A). In addition, serum sTNF-RI was significantly elevated in HIVL+ compared with HIVL− (P < 0.05), and serum sTNF-RII tended to be elevated in HIVL+ compared with HIVL− (P < 0.06), but only in patients undergoing HAART (Fig. 4A). Serum IL-6 was greater in HIVL+ compared with the respective HIVL− groups, in both HAART+ and HAART− (Fig. 4A).

Serum leptin was not significantly different by HAART status when examined separately for HIVL+ and HIVL− (Fig. 4B); however, it was lower overall in HAART+ compared with HAART− (P < 0.05) in all HIV-infected subjects (not shown). Additionally, serum leptin (and insulin, not shown) was significantly elevated in HIVL+ compared with HIVL−, but only in the HAART+ groups (Fig. 4B).

Serum lactate was measured in only a small number of subjects and was significantly elevated in HAART+ compared

### Table 3. Therapeutic regimes of HIVL+ and HIVL− subjects

<table>
<thead>
<tr>
<th></th>
<th>HIVL+(n = 27)</th>
<th>% of total</th>
<th>HIVL−(n = 16)</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any antiviral</td>
<td>22</td>
<td>81</td>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>NRTI only</td>
<td>1</td>
<td>3.8</td>
<td>1</td>
<td>5.8</td>
</tr>
<tr>
<td>NRTI + PI</td>
<td>12</td>
<td>46</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>NRTI + NNRTI</td>
<td>6</td>
<td>22</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>NRTI + PI + NNRTI</td>
<td>3</td>
<td>11.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-NRTI; PI, protease inhibitors.

Circulating IL-6 levels were positively correlated with VAT (P = 0.001) and negatively correlated with the percentage of fat in the legs (P < 0.02, %Leg Fat and %Leg SAT; Table 2). Serum sTNF-RI and -RII also tended to be positively associated with VAT and negatively associated with %Leg SAT, but these correlations were not statistically significant (P = 0.06). Serum leptin was positively associated with SAT but not with VAT (P = 0.4; not shown). There were no significant relationships of serum cytokine release with body composition or body fat distribution (not shown).

IL-6 secretion from abdominal SAT was positively associated with the total amount of VAT (P = 0.02; Table 2). TNF-α release was also positively related to VAT, but this did not reach statistical significance (P = 0.06). Additionally, adipose tissue release of both TNF-α and IL-6 was negatively correlated with %Leg SAT (P < 0.01; Table 2). There were no significant relationships of abdominal adipose tissue lactate or glycerol release with body composition or body fat distribution (not shown).

We also examined the relationships of serum insulin to circulating inflammatory markers in HIV-infected subjects (not shown). Serum insulin was positively associated with serum leptin levels (P = 0.03) and tended to be positively associated with serum levels of IL-6 and sTNF-RI, but these did not reach statistical significance (P = 0.06 and P = 0.07, respectively).

Relationship of HAART to body composition, circulating proinflammatory markers, and adipose tissue metabolism in HIV-infected subjects. Of the 43 HIV-infected subjects studied, 32 were undergoing some form of antiretroviral therapy (Table 3). More HIVL+ patients were undergoing HAART (defined as taking NRTI or PI alone or in any combination) than were HIVL− patients (81 vs. 63%). Additionally, more HIVL+ patients were taking PI than were HIVL− (58 vs. 29%), but none of these differences was statistically significant. We examined the viral loads (HIV RNA levels) and CD4 counts for these HIV-infected patients from their clinical charts (not shown). The viral loads were not different between HIVL+ and HIVL−, whereas the CD4 count was greater in the HIVL− than in the HIVL+ group (577 ± 279 vs. 358 ± 222 cells/μL, P = 0.02).

Overall, there were no significant differences in BMI, percent body fat, WHR, SAT, and VAT between those subjects undergoing HAART and those who were not. However, fat redistribution by DEXA (lower %Leg Fat and higher %Trunk Fat) was specifically observed in those subjects taking PI compared with those not taking PI (31.4 ± 10 vs. 23.1 ± 12%, P < 0.06 for %Leg Fat; 63.7 ± 13 vs. 54.1 ± 9%, P < 0.05 for %Trunk Fat, respectively).
with HAART−, but only in HIVL+ (Fig. 4C). Furthermore, lactate was elevated in subjects taking PI compared with those taking other medications or on no treatment (2.2 ± 0.6 vs. 1.1 ± 0.2 ng/ml, \(P < 0.001\)) and in subjects taking NRTI compared with those not taking NRTI (1.9 ± 0.7 vs. 1.0 ± 0.2 ng/ml, \(P < 0.01\)).

The metabolic characteristics of abdominal SAT are shown in Fig. 5. The mean cell size of abdominal SAT was similar in HIVL+ and HIVL− in the HAART− groups, but it was greater in HIVL+ compared with HIVL− in the HAART+ groups (Fig. 5A). In addition, release of TNF-α from abdominal SAT was increased in HIVL+ compared with HIVL− but only in the HAART+ groups (Fig. 5B). No statistically significant differences between groups were found in the secretion of IL-6 and leptin from adipose tissue (Fig. 5, B and C), although the pattern was similar to that seen for the circulating levels (see Fig. 4, A and C). Lactate release from abdominal SAT was elevated in HAART+ compared with HAART−, but this difference was significant only in HIVL+ (Fig. 5D). Glycerol release paralleled lactate release, with a tendency for HAART+ to have greater lipolysis than HAART− (Fig. 5D).

We further analyzed the adipose tissue metabolic data separately for HIV-infected subjects taking NRTI or PI compared with those who were not taking these medications. HIV-infected subjects taking NRTI (in any combination) had decreased adipose tissue leptin release (39.9 ± 22.3 vs. 57.6 ± 21.6 ng·g⁻¹·h⁻¹, \(P < 0.05\)) and marginally greater adipose tissue lactate release (3,500 ± 1,682 vs. 2,313 ± 881 nmol·g⁻¹·h⁻¹, \(P = 0.055\)) compared with those not taking NRTI. However, neither leptin nor lactate release from abdominal SAT was specifically related to PI therapy.

**DISCUSSION**

The etiology and pathophysiology of the fat redistribution, insulin resistance, and dyslipidemia found in HIV-infected patients since the emergence of HAART are incompletely understood (32, 52, 68). HAART has been implicated in the pathogenesis of HIV-associated lipodystrophy (10), but fat redistribution can occur in patients who are not undergoing HAART (27, 38, 41, 52).

Host factors such as immune dysregulation may play a role in the development of lipodystrophy (11, 41). Increased circulating levels of sTNF-RII and increased TNF-α expression in abdominal SAT have been found in HIV-infected patients with peripheral lipodystrophy (3, 50). However, adipose tissue secretion of TNF-α and other inflammatory markers, and their relationships to changes in body fat distribution, have not been examined before in the HIV-lipodystrophy syndrome.

In this study, we compared adipose tissue cytokine secretion and other aspects of adipose tissue metabolism (leptin, glycerol, and lactate release), as well as circulating levels of proinflammatory markers, in HIV-infected subjects with and without lipodystrophy. We defined lipodystrophy clinically as a significant change in body habitus observed by both the HIV patient and his/her clinician. Using objective measurements (DEXA and whole body MRI), we found significantly lower absolute amounts of lower limb adipose tissue (Leg Fat and Leg SAT) in HIV-infected compared with control subjects. Moreover, the HIVL+ group had a greater accumulation of fat in the upper body (VAT and Trunk SAT) and a lesser relative accumulation of fat in the lower periphery (%Leg Fat and %Leg SAT) compared with the HIVL− subjects. Thus we were able to identify specific objective indexes of fat redistribution in HIVL+ that were independent of the overall adipose tissue loss observed in the HIV-infected subjects compared with controls.

We have shown that adipose tissue metabolism is altered in several ways in HIVL+ subjects. These metabolic changes are paralleled by changes in circulating inflammatory markers and in other metabolites such as lactate. The secretion of TNF-α from abdominal SAT, as well as the circulating levels of IL-6 and sTNF-RII, were elevated in HIV-infected subjects with fat redistribution. Increased adipose tissue production and circulating levels of cytokines, in particular IL-6, were specifically associated with increased VAT and with subcutaneous fat redistribution (decreased %Leg Fat and %Leg SAT) in the HIV-infected subjects.

The hypercytokinemia in the HIVL+ patients could represent an overall upregulation of the TNF-α system. HIV infection is in itself associated with elevated cytokines, which in turn may be partly responsible for the hypertriglyceridemia seen in these patients (25). Additionally, levels of circulating soluble TNF-α receptors may act as markers for the clinical course of HIV infection (24). When HAART is effective, HIV-infected patients have reduced components of the TNF-α system along with decreased mortality, lower viral loads, and increased CD4 counts (2, 28, 53). However, dysregulation of the TNF-α system may occur along with immune improvement after initiation of HAART, including the accumulation of T.
cells primed for TNF-α secretion (4, 40). Individual variability in cytokine response, such as produced by a TNF-α gene polymorphism, may further play a role in the development of lipodystrophy during HAART (44).

In non-HIV subjects, increased adipose tissue TNF-α production and circulating levels of IL-6, sTNF-RI, and sTNF-RII are associated with obesity and insulin resistance (6, 7, 16, 30, 37). Increased TNF-α secretion from adipose tissue is believed to produce elevated systemic IL-6 (36, 47) and lead to enhanced shedding of sTNF-RII receptors from the adipose tissue (46). Elevated TNF-α secretion from adipose tissue can also produce local and systemic insulin resistance, with a compensatory increase in circulating insulin levels (20, 31, 39, 55, 64, 65). Hyperinsulinemia may in itself further contribute to enlarged upper body adipose tissue depots (including visceral) and increased systemic IL-6 production, thus promoting a vicious cycle. Consistent with these potential effects of TNF-α, we observed elevated insulin and IL-6 levels in the HIVL+ group compared with HIVL− and control groups. However, we did not measure systemic insulin sensitivity in our subjects, which is a major limitation of our study.

Whether our observed correlations between increased adipose tissue cytokine production and fat redistribution (increased VAT and decreased lower limb fat) in HIV-infected subjects represent a cause or an effect cannot be determined from this cross-sectional study. However, increased TNF-α production may potentially change the size of the subcutaneous depots by several means: by increasing lipolysis and decreasing LPL activity (20, 55); by decreasing lipogenic enzymes such as acylation-stimulating protein (Ionescu G, unpublished data); by downregulating the adipogenic differentiation factors CCAAT enhancer-binding protein-α, peroxisome proliferator-activated receptor-γ, and sterol regulatory element-binding protein isoform 1c (3, 64); or by increasing adipose tissue apoptosis (13). Additionally, IL-6 may also play a role in accelerating lipodystrophy by enhancing lipolysis, as has been shown to occur in cancer cachexia and other wasting syndromes (21).

Alternatively, enhanced adipose tissue cytokine production may instead be a consequence, rather than a cause, of the fat redistribution. We have previously shown regional differences in IL-6 and TNF-α secretion in obese non-HIV patients, with in vitro production of these cytokines being greater in visceral (omental and mesenteric) fat than in abdominal SAT (18, 33). We have shown here that the HIVL+ group had a greater amount of VAT than did the group without lipodystrophy and that circulating IL-6 was proportional to the amount of VAT in the HIV-infected subjects, suggesting that the excess visceral fat in HIVL+ may have contributed to the increased serum IL-6 in these subjects. However, fat redistribution cannot be the sole explanation for the altered cytokine production in HIVL+, because the HIVL+ subjects had similar visceral and truncal adiposity compared with controls but had elevated IL-6 and sTNF-RII levels.

We also observed regional heterogeneity in cytokine and metabolite secretion across regions of SAT (DC vs. abdominal) in the HIVL+ patients with enlarged buffalo humps, which is a novel finding of our study. We found that significantly less lactate and TNF-α, and somewhat less glycerol, was secreted from DC fat compared with abdominal SAT. Therefore, we believe that DC fat is not a major contributor to the increased circulating levels of proinflammatory markers observed in HIVL+ subjects.

Typically, extra deposition of DC adipose tissue occurs during corticosteroid excess such as that seen in Cushing's syndrome. This suggests that DC fat may metabolically resemble omental adipose tissue, which has a greater density of glucocorticoid receptors and is more responsive to cortisol than is abdominal SAT (54, 61). However, TNF-α and IL-6 production from DC fat, unlike VAT, does not appear to be greater than that in abdominal SAT (18, 33). Overall, our speculation is limited by the fact that we did not examine cytokine release in VAT from HIV-infected patients.

Basal lipolysis (glycerol release per gram adipose tissue) was increased in abdominal SAT from HIV-infected subjects, paralleling the increased systemic NEFA flux reported by others in this patient population (26). However, in general, glycerol release was not related to adipose tissue TNF-α secretion and was only modestly related to IL-6 release. Therefore, increased basal lipolysis may not play a major role in the mechanisms relating increased TNF-α production to fat redistribution (6, 55). Alternatively, our cross-sectional design may obscure the longitudinal relationship of TNF-α to lipolysis in HIV infection, since lipolysis increases early on with the development of wasting and then abates somewhat when fat cell size decreases. Moreover, it should be noted that measurements of basal lipolysis in adipose tissue fragments are rather crude and do not allow for the control of local lipolytic regulatory factors such as adenosine and prostaglandins (35).

Still, because adipose tissue lipolysis is known to be correlated with adipocyte size in non-HIV subjects (34), it is surprising that the basal lipolysis was increased in the HIVL− relative to controls despite their smaller adipocyte size. This may represent a dissociation between adipocyte size and glycerol release in HIV-infected subjects as a consequence of HAART. PI increase lipolysis and induce insulin resistance in adipocytes (8, 14, 48, 49, 51, 57, 60, 70). In contrast, NRTI reduce adipocyte size and are thought to decrease lipolysis (52). In our patients, there was a tendency for glycerol release to be greater, albeit not significantly so, in HIV-infected patients undergoing HAART compared with those not on treatment.

We also have shown an increased lactate secretion from SAT in association with HAART in HIV-infected subjects, and this finding was stronger in the HIVL+ group. Increased systemic lactate levels were previously reported in HIV patients undergoing HAART (52). Adipose tissue mitochondrial damage from HAART with subsequent increased lactate production was hypothesized as the most likely cause of hyperlactatemia in these patients (12, 45, 63, 67), as liver lactate clearance was found to be normal (58). Our results suggest that adipose tissue is indeed one possible source of the increased circulating lactate, but this does not preclude increased lactate production from other tissues such as muscle.

Overall, circulating leptin levels in our subjects reflected the amount of total body fat, since leptin was highly correlated with adiposity in all groups. This confirms previous reports that HIV-infected subjects do not have changes in circulating leptin beyond those predicted by changes in fat mass (69). In this regard, higher circulating leptin levels in HIVL+ compared with HIVL− were probably related to the overall decrease in truncal adipose tissue in the HIVL− subjects. However, sep-
oration of the groups by HAART status revealed a subtle, although not significant, decrease in adipose tissue production of leptin. For NRTI treatment specifically, adipose tissue leptin secretion was significantly decreased. This may have contributed to the decreased leptin levels in HIV patients undergoing HAART compared with those not on treatment (HAART–), as no significant differences in adiposity and overall fat mass were found between these groups.

The metabolic effects of PI and NRTI have been documented in vitro and in vivo in animal models (52); however, their effect on cytokine production has not been reported. Our attempts to separate the effects of HAART and those of fat redistribution, with regard to proinflammatory cytokine production and circulating levels, were limited by the small number of patients not undergoing HAART in our study groups. Circulating levels of sTNF-RI were increased with HAART, but this was true only for the HIVL+ subjects. In addition, elevations in adipose tissue cytokine production (TNF-α) and serum levels of proinflammatory cytokines (sTNF-RI, sTNF-RII, and IL-6) observed in HIVL+ with respect to HAART+ were more pronounced in patients undergoing HAART. The lack of significant differences for cytokines in the HAART– groups may have been due to the small subject number in these groups.

Carr et al. (10) have proposed that two separate lipodystrophy syndromes exist, one resulting from NRTI treatment, associated primarily with lipoatrophy and potential mitochondrial dysfunction, and the other resulting from PI treatment, associated primarily with accumulation of visceral and DC adipose tissue. With regard to this hypothesis, our findings suggest that increased production of proinflammatory cytokines and HAART may both play a role in causing adipose tissue redistribution and metabolic abnormalities (e.g., hyperinsulinemia) in the HIV-lipodystrophy syndrome and that, additionally, there may be a synergistic deleterious effect of HAART with proinflammatory cytokines (particularly TNF-α) on adipose tissue metabolism (48), resulting in fat redistribution and other manifestations of the metabolic syndrome.

In summary, we showed that HIV-infected subjects with fat redistribution (increased visceral fat and relatively decreased lower body subcutaneous fat) have increased adipose tissue TNF-α secretion and circulating levels of proinflammatory markers. We note that these increases were more pronounced in patients undergoing HAART, with the caveat that our sample of HAART– patients was quite small. In the same context, decreased leptin and increased lactate secretion in adipose tissue were specifically related to HAART. We conclude that increased adipose tissue production and serum levels of proinflammatory cytokines may play a significant role in the occurrence of the fat redistribution/metabolic syndrome in the HIV-infected patients undergoing HAART.

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