# Human Immunodeficiency Virus Type 1–Related Lipoatrophy and Lipohypertrophy Are Associated with Serum Concentrations of Leptin

**G. Sonia Nagy,[1] Sotirios Tsiodras,[1] Lizabeth D. Martin,[1] Anchalee Avihingsanon,[1] Alina Gavrila,[2] William C. Hsu,[2] Adolf W. Karchmer,[1] and Christos S. Mantzoros[2]**

Divisions of [1]Infectious Diseases and [2]Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

**The relationship between the adipocyte-derived hormone leptin, insulin resistance, and fat redistribution in patients with human immunodeficiency virus (HIV) infection has not been established. We classified a cohort of HIV type 1 (HIV-1)–infected patients with ⩾6 months of antiretroviral exposure as having no lipodystrophy (51 patients [43% of the cohort]), lipoatrophy (23 patients [19% of the cohort]), mixed lipodystrophy (29 patients [24% of the cohort]), or lipohypertrophy (17 patients [14% of the cohort]), on the basis of physical examination, anthropometric measurements, and the findings of dual-emission x-ray absorptiometry and computed tomography. Measurements of insulin resistance were higher for patients with each category of lipodystrophy, compared with those observed for patients with no lipodystrophy ($P < .001$). Mean leptin levels ($\pm$ standard deviation) were lowest in patients with lipoatrophy (1.76 $\pm$ 1.20 ng/mL), highest in patients with lipohypertrophy (9.10 $\pm$ 6.86 ng/mL), and significantly different from those in patients without lipodystrophy (3.14 $\pm$ 2.30 ng/mL; both $P < .01$). In this cohort of antiretroviral-experienced HIV-infected patients, a low serum level of leptin was independently associated with insulin resistance in patients with lipoatrophy, after controlling for total and regional body fat.**

Leptin is the hormone product of the obese *(ob)* gene, synthesized in and secreted from adipocytes. This hormone is involved in energy homeostasis and insulin resistance via central effects on the hypothalamus and peripheral effects on fatty acid oxidation [1–5]. In patients without HIV infection, fasting serum concentrations of leptin correlate positively with total and central fat accumulation and are associated with insulin resistance [6–8]. Leptin deficiency or leptin resistance due to mutations of the leptin receptor in rodents can lead to hyperinsulinemia and the development and persistence of obesity and insulin resistance. In addition, exogenous leptin administration can restore insulin sensitivity in leptin-deficient lipoatrophic mice, independently and before the occurrence of any changes in the body weight of such mice [9, 10]. In humans, both congenital lipoatrophy and acquired lipoatrophy are characterized by a generalized loss of subcutaneous and visceral fat, insulin resistance, and hyperlipidemia [11–13]. Recently, it was demonstrated that leptin replacement in adults with acquired and congenital lipodystrophy syndromes resulted in improved glycemic control and decreased triglyceride levels, allowing for the decrease or discontinuation of antidiabetic therapy [14].

The use of HAART for HIV infection has resulted

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Reprints or correspondence: Dr. Christos S. Mantzoros, Div. of Endocrinology, Research N. 325, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215 (cmantzor@caregroup.harvard.edu).

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in remarkable decreases in morbidity and mortality in this patient population as a result of the suppression of viral replication. Because patients with HIV infection are living longer, however, body fat distribution abnormalities and metabolic derangements are becoming increasingly apparent. Estimates of the prevalence of lipodystrophy syndrome are as high as 84% for patients taking protease inhibitors (PIs) and 41% for PI-naive patients [15–18]. As with patients with congenital lipodystrophy syndromes, patients with HIV lipodystrophy also manifest various metabolic abnormalities. Lipid abnormalities (elevated total cholesterol, triglyceride, and low-density lipoprotein [LDL] cholesterol levels and a decreased high-density lipoprotein [HDL] cholesterol level) are seen in up to 60% of patients treated with PIs [19, 20], whereas impaired glucose tolerance occurs in up to 47% of such patients [21, 22].

Given the similarities between the metabolic complications observed in patients with HIV-related lipodystrophy and those seen in patients with other lipodystrophy syndromes, we sought to investigate further the relationship between leptin and the HIV-related fat redistribution syndrome. To date, there have been a small number of studies reporting leptin levels in HIV-infected patients with lipodystrophy. These studies provided conflicting findings regarding the relationship between leptin and fat redistribution, but they did not evaluate subjects with different patterns of fat redistribution separately [1, 23–28]. We evaluated the association between leptin, the various phenotypic manifestations of lipodystrophy, and the metabolic profiles while controlling for several potential confounders, including regional and total body fat, exercise, the $CD4^+$ T cell count at baseline, and the use of HAART.

## PATIENTS AND METHODS

***Study population.*** Patients were recruited from 2 ambulatory care HIV clinics at an urban academic medical center and were eligible for inclusion if, at the time of the study, they were infected with HIV, were >16 years of age, and had received ⩾6 months of cumulative exposure to any antiretroviral regimen (PI-experienced and PI-naive patients were included). The study was approved by the Committee on Clinical Investigation (CCI) of the Beth Israel Deaconess Medical Center (Boston, MA) and was conducted with the written consent of each participant. Guidelines for human experimentation from the US Department of Health and Human Services (Washington, DC) and the CCI of Beth Israel Deaconess Medical Center were followed in the conduct of this research project.

***Study visit.*** One hundred twenty-six patients were interviewed using a detailed questionnaire administered by one of the study investigators, and medical records were reviewed to confirm the information reported by the patients. Patients were examined, and anthropometric measurements (of skin folds, the waist-to-hip ratio, body mass index (BMI), and the widest diameter of the "buffalo hump," when present) were made in a standardized fashion. Digital photographs of predetermined views (i.e., the face, gluteus region, legs, arms, and chest, as well as a full side view) were taken for documentation of body fat distribution. A fat redistribution adjudication committee that consisted of investigators not involved in the patient interviews, data collection, or data analysis reviewed the standard photographs of each patient, together with the anthropometric measurements, and they then classified the patients as belonging to 1 of 4 subgroups: the no lipodystrophy group (i.e., the No LD group), the lipoatrophy group (i.e., the LA group; patients had subcutaneous fat loss involving the face, gluteus region, and extremities), the lipohypertrophy group (i.e., the LH group; patients had fat accumulation consisting of a buffalo hump, central adiposity, or multiple lipomas), or the mixed lipodystrophy group (i.e., the MLD group; patients had features of both lipoatrophy and lipohypertrophy) [29]. Adjudication was conducted by following strict criteria outlined within the study protocol.

***Laboratory analysis.*** On the day of the study visit, fasting blood samples were obtained to measure leptin, insulin, glucose, and lipid levels. Leptin and insulin levels were assayed by commercially available radioimmunoassay (RIA) kits (manufactured by Linco Research [for assessment of leptin levels] and DSL [for assessment of insulin levels]). The lower limits of detection of the assays were 0.5 ng/mL for leptin and 1.3 $\mu$IU/mL for insulin. The intra-assay coefficients of variation were 5%–8% for leptin and 4.5%–8.3% for insulin. Current data on the $CD4^+$ T cell count and the HIV load were extracted from the medical record by use of values obtained within 4 weeks of the study visit. The $CD4^+$ T cell count at the time of diagnosis of HIV infection was obtained from the patients' electronic medical records. Insulin resistance was determined using the homeostasis model assessment index (HOMA-IR) with the following formula: (fasting insulin level in microunits per milliliter $\times$ glucose level in millimoles per liter)/22.5. Fasting lipoprotein fractions were measured as described elsewhere [30, 31]. Plasma and lipoprotein fractions were assayed for total cholesterol and triglyceride by use of an Abbott Spectrum analyzer with the use of enzymatic reagents [32]. HDL cholesterol was measured as described elsewhere [30]. The level of LDL cholesterol was calculated with the Friedewald equation when triglyceride levels were <400 mg/dL. For subjects with triglyceride levels of >400 mg/dL, a direct LDL assay was used to calculate the LDL cholesterol level. Lipid assays were standardized through the Centers for Disease Control and Prevention (Atlanta, GA) Lipid Standardization Program.

***Radiological studies.*** Dual-emission x-ray absorptiometry (DEXA; Hologic 2000) scans were obtained to determine fat and fat-free soft-tissue masses for the whole body, abdomen,

and extremities. A single-slice CT image obtained at the level of L4 was obtained to examine the intra-abdominal adipose tissue and the subcutaneous abdominal adipose tissue.

***Data analysis.*** Data were analyzed using SAS software, version 8.2 (SAS Institute). Results are reported as mean ± SD. The Kruskal-Wallis test was used for the analysis of continuous variables between the 4 groups, such as demographic and anthropometric data, data on antiretroviral use, and immunologic, virologic, and metabolic parameters. Post hoc comparisons between subgroups were done using the Wilcoxon rank sum test for 4 prespecified comparisons: the No LD group versus the LA group, the No LD group versus the MLD group, the No LD group versus the LH group, and the LA group versus the LH group. The *P* values for these comparisons were multiplied by 4 to adjust for multiple comparisons.

For analysis of categorical variables (demographic characteristics and antiretroviral drug use), the $\chi^2$ test was used, and, for post hoc subgroup analysis, Fisher's exact test was used, with adjustment of *P* values as described above. Spearman correlation coefficients were derived for each lipodystrophy subgroup to evaluate the relationship between leptin levels and the other metabolic laboratory values measured (i.e., total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, glucose, and insulin levels and HOMA-IR). Multiple linear regression analysis was performed to evaluate leptin as a predictor of lipid, glucose, and insulin levels and HOMA-IR, after adjustment for age, sex, exercise, total nucleoside reverse-transcriptase inhibitor (NRTI) use (i.e., the cumulative duration of use of all NRTIs, in months), and $CD4^+$ T cell count at diagnosis of HIV infection. Logistic regression analysis was performed, with adjustment for the same independent variables, to assess whether leptin was an independent predictor of patients belonging to one of the lipodystrophy subgroups versus the No LD group. For linear and logistic regression analysis, variables were included if they were found to be statistically significant in univariate analysis and if they were thought to be associated with lipodystrophy. For all analyses, $P < .05$ was considered to be statistically significant.

## RESULTS

***Baseline demographic and anthropometric characteristics of study subjects.*** One hundred twenty-six patients with HIV infection were enrolled in the study; 120 of the 126 patients were adjudicated into 1 of 4 body fat redistribution categories: 51 (43%) were in the No LD group, 23 (19%) were in the LA group, 29 (24%) were in the MLD group, and 17 (14%) were in the LH group (table 1). Six patients who could not be adjudicated because of a lack of consensus within the adjudication committee were not included in the analyses.

When analyses of the baseline variables according to lipodystrophy subgroup were performed, there were a number of significant differences. There were differences in sex distribution between the 4 groups ($P < .05$), with there being a higher percentage of men in the LA group (22 patients [96%]). The group with LH had the highest percentage of women (5 patients [29%]), compared with the MLD group (4 patients [14%]) and the LA group (1 patient [4%]) ($P < .05$). Race/ethnicity differed between the 4 groups included in the cohort: 105 patients (87.5%) were white, 8 (6.7%) were Hispanic, and 7 (5.8%) were African American ($P = .04$; data not shown). However, analysis of subgroups according to lipodystrophy category did not reveal any significant differences in race/ethnicity. There was a statistically significant difference in the mean age of the patients ($P < .05$) and in the amount of exercise between groups ($P < .01$). In the overall analysis, there was no difference with regard to current or past smoking status (yes/no), total tobacco use (i.e., the number of packs of cigarettes smoked per week multiplied by number of years that the subjects smoked), extent of current alcohol use (i.e., number of drinks per week), coinfection with hepatitis B or C (defined as a positive result of a hepatitis B surface antigen test or hepatitis C antibody test), or duration since diagnosis of HIV infection (number of months) (data not shown).

With regard to virologic and immunologic characteristics, there was a statistically significant difference between subgroups regarding the $CD4^+$ T cell count at diagnosis of HIV infection, such that patients in the LH and MLD groups had lower $CD4^+$ T cell counts at diagnosis (287 ± 214 and 285 ± 191 cells/$\mu$L, respectively), and such that patients in the LA and No LD groups had higher $CD4^+$ T cell counts at diagnosis (444 ± 344 and 448 ± 262 cells/$\mu$L, respectively) ($P < .05$) (table 1). Post hoc subgroup analysis revealed that the difference in the $CD4^+$ T cell count at diagnosis was significant only for the MLD group, compared with the No LD group ($P < .05$). There was no difference between subgroups with regard to the nadir $CD4^+$ T cell count, the $CD4^+$ T cell count at the time of the study visit, and the HIV load (as measured by branched DNA assay) at the time of the study visit. There was a statistically significant difference in total NRTI use (total duration of use of all NRTIs [in months]) between the 4 groups: patients in the No LD group used NRTIs for the least amount of time (96 ± 58 months), patients in the LH group used NRTIs for an intermediate length of time (104 ± 52 months), and patients in the MLD and LA groups had the highest cumulative duration of exposure to NRTIs (148 ± 45 months and 146 ± 49 months, respectively) ($P < .001$). This difference remained significant in the post hoc subgroup analyses of the LA group versus the No LD group and of the MLD group versus the No LD group ($P < .01$ for each test). There was no difference between the groups with regard to total PI or nonnucleoside reverse-transcriptase inhibitor (NNRTI) use or to having ever used (yes/

**Table 1. Baseline characteristics of or findings for 120 HIV-infected patients with ⩾6 months of antiretroviral exposure, by lipodystrophy subgroup classification.**

| Characteristic or finding | No LD group ($n$ = 51) | LA group ($n$ = 23) | MLD group ($n$ = 29) | LH group ($n$ = 17) | Overall $P$ |
|---|---|---|---|---|---|
| Male sex, no. (%) of patients | 48 (94) | 22 (96) | 25 (86) | 12 (71) | <.05 |
| Age, years | 42 ± 8 | 44 ± 7 | 47 ± 9[a] | 43 ± 7 | <.05 |
| Total exercise[b] | 807 ± 664 | 910 ± 719 | 379 ± 449[a] | 621 ± 659 | <.01 |
| $CD4^+$ T cell count at diagnosis of HIV infection, cells/μL | 448 ± 262 | 444 ± 344 | 285 ± 191[a] | 287 ± 214 | <.05 |
| Total duration of use of all NRTIs, months | 96 ± 58 | 146 ± 49[c] | 148 ± 45[c] | 104 ± 52 | <.001 |
| Body mass index | 23.96 ± 2.43 | 22.34 ± 2.60 | 24.75 ± 2.91 | 28.34 ± 4.81[c,d] | <.0001 |
| Body weight, kg | 73.3 ± 10.0 | 68.9 ± 9.7 | 75.0 ± 13.1 | 84.0 ±15.5[a,e] | <.05 |
| Percentage of total body fat | 19.4 ± 6.3 | 12.1 ± 4.8[c] | 20.2 ± 7.6 | 28.6 ± 8.4[c,d] | <.0001 |
| DEXA findings | | | | | |
| Abdominal fat | 19.9 ± 7.1 | 12.4 ± 7.2[c] | 23.9 ± 8.6 | 31.8 ± 23.6[c,d] | <.0001 |
| Lower limb fat | 18.5 ± 8.3 | 9.6 ± 4.5[c] | 13.2 ± 8.4[c] | 23.6 ± 8.7[d] | <.0001 |
| Ratio of abdominal fat to lower limb fat | 1.2 ± 0.5 | 1.4 ± 0.8 | 2.0 ± 0.6[c] | 1.4 ± 0.5 | <.0001 |
| Total fat area, $cm^2$ | 260.9 ± 90.6 | 174.1 ± 84.3[c] | 341.6 ± 131.9 | 483.4 ± 183.5[c,d] | <.0001 |
| Abdominal fat, $cm^2$ | | | | | |
| Subcutaneous | 122.9 ± 66.2 | 47.1 ± 33.0[c] | 118.0 ± 88.4 | 247.1 ± 105.5[c,d] | <.0001 |
| Visceral | 98.0 ± 43.1 | 90.8 ± 52.7 | 143.8 ± 110.6[c] | 181.3 ± 81.1[c,d] | <.0001 |
| Percentage of visceral fat | 37.7 ± 13.5 | 50.8 ± 12.3[c] | 43.6 ± 30.7 | 38.6 ± 10.9[e] | <.01 |
| Waist-to-hip ratio | 0.95 ± 0.05 | 0.92 ± 0.04 | 1.00 ± 0.05[c] | 1.02 ± 0.05[c,d] | <.0001 |
| Cholesterol level, mg/dL | | | | | |
| Total | 212.00 ± 55.65 | 210.59 ± 54.85 | 225.89 ± 93.08 | 210.47 ± 47.29 | NS[f] |
| LDL | 125.56 ± 44.20 | 116.16 ± 32.80 | 104.43 ± 44.90 | 122.47 ± 40.37 | NS |
| HDL | 39.20 ± 10.85 | 30.01 ± 9.15[c] | 30.71 ± 9.53[c] | 41.79 ± 13.04[e] | <.0001 |
| Triglyceride level, mg/dL | 218.82 ± 157.39 | 353.05 ± 234.07[a] | 530.75 ± 618.65[c] | 231.53 ± 191.69 | <.001 |
| Glucose level, mg/dL | 84.80 ± 9.00 | 86.64 ± 15.07 | 96.93 ± 43.13 | 115.25 ± 53.35 | NS |
| Insulin level, μU/mL | 12.46 ± 6.03 | 26.40 ± 30.80[c] | 31.55 ± 28.40[c] | 30.58 ± 24.12[c] | <.0001 |
| HOMA-IR[g] | 2.55 ± 1.28 | 5.62 ± 5.89[c] | 8.84 ± 11.44[c] | 10.09 ± 10.75[c] | <.001 |
| Leptin level, ng/mL | 3.14 ± 2.30 | 1.76 ± 1.20[c] | 4.88 ± 4.90 | 9.10 ± 6.86[c,d] | <.0001 |

**NOTE.** Data are mean ± SD, unless otherwise indicated. DEXA, dual-emission x-ray absorptiometry; HDL, high-density lipoprotein; HOMA-IR, insulin resistance determined using the homeostasis model assessment index; LA group, patients with lipoatrophy (19% of the cohort); LDL, low-density lipoprotein; LH group, patients with lipohypertrophy (14% of the cohort); MLD group, patients with mixed lipodystrophy (peripheral lipoatrophy with central fat accumulation; 24% of the cohort); No LD group, patients with no evidence of lipodystrophy (43% of the cohort); NRTI, nucleoside reverse-transcriptase inhibitor; NS, not significant.

[a] $P < .05$ compared with the No LD group, by post hoc analysis.

[b] See sentences 2–4 of paragraph 3 of the "Metabolic and hormonal measurements" subsection of the Results section for information on how total exercise was determined.

[c] $P < .01$ compared with the No LD group, by post hoc analysis.

[d] $P < .01$ compared with the LA group, by post hoc analysis.

[e] $P < .05$ compared with the LA group, by post hoc analysis.

[f] $P = .05$.

[g] HOMA-IR formula: (fasting insulin level in microunits per milliliter × glucose level in millimoles per liter)/22.5

no) or currently using (yes/no) PIs, NNRTIs, or NRTIs (data not shown). When cumulative use of individual PIs was analyzed (as defined by cumulative treatment [in months]) for each of the following: amprenavir, indinavir, nelfinavir, full-dose ritonavir, booster-dose ritonavir, all ritonavir use, saquinavir, and lopinavir), only saquinavir use was found to be significantly different between lipodystrophy groups (mean duration ± SD, 3.33 ± 8.07 months for the No LD group, 10.48 ± 16.80 months for the LA group, 9.69 ± 12.55 months for the MLD group, and 8.24 ± 14.58 months for the LH group; $P = .03$ for overall comparison; data not shown). Post hoc testing that compared subgroups found a statistically significant

difference in total saquinavir use between only the No LD and MLD groups ($P = .02$).

There were statistically significant differences between the 4 lipodystrophy groups with regard to anthropometric and physical (body fat distribution) characteristics, findings that would be expected (given our subclassification of patients), and findings that affirmed the adjudication of subjects appropriately into each of the 4 subgroups (table 1). Specifically, there were differences between the groups with regard to BMI; the percentage of total body fat; abdominal fat and lower limb fat (as determined by DEXA); the ratio of abdominal fat to lower limb fat (as determined by DEXA); total, abdominal subcutaneous, and abdominal visceral fat (as determined by CT); and the waist-to-hip ratio ($P < .0001$ for all of these measures); there were also differences regarding the percentage of visceral fat (as determined by CT) ($P < .01$) and weight ($P < .05$). Although patients in the LA group had the lowest percentage of total body fat, total fat area, total abdominal subcutaneous fat, and total abdominal visceral fat, they had the highest percentage of visceral fat (50.8% ± 12.3% vs. 37.7% ± 13.5% for the No LD group, 43.6% ± 30.7% for the MLD group, and 38.6% ± 10.9% for the LH group; $P < .01$). Post hoc analyses of these findings revealed that these differences remained significant for the LA group versus the No LD group (percentage of total body fat, abdominal fat and lower limb fat [as determined by DEXA], total fat area, abdominal subcutaneous fat, and percentage of visceral fat), for the LH group versus the No LD group (BMI, weight, percentage total body fat, abdominal fat [as determined by DEXA], total fat area, abdominal subcutaneous fat, abdominal visceral fat, and the waist-to-hip ratio), for the MLD group versus the No LD group (lower limb fat [as determined by DEXA], ratio of abdominal fat to lower limb fat [as determined by DEXA], abdominal visceral fat, and the waist-to-hip ratio), and for the LH group versus the LA group (BMI, weight, percentage total body fat, abdominal fat and lower limb fat [as determined by DEXA], total fat area, abdominal subcutaneous fat, abdominal visceral fat, percentage of visceral fat, and the waist-to-hip ratio).

***Metabolic and hormonal measurements.*** There were statistically significant differences between the 4 subgroups regarding the levels of HDL cholesterol, triglycerides, and insulin, and, also, HOMA-IR (table 1). Patients in the LA and MLD groups had the lowest HDL levels (30.01 ± 9.15 mg/dL and 30.71 ± 9.53 mg/dL, respectively; $P < .0001$ overall; $P < .01$ for analysis of the LD subgroups vs. the No LD group), and patients in all 3 LD groups (LA, MLD, and LH) had higher insulin levels and HOMA-IR, compared with those of patients in the No LD group ($P < .001$ overall and $P < .01$ for each subgroup analysis). There was also a statistically significant difference in leptin levels between the 4 subgroups: patients in the LA group had the lowest leptin levels (1.76 ± 1.20 ng/mL), and patients in the LH group had the highest leptin levels (9.10 ± 6.86 ng/mL) ($P < .0001$ overall; $P < .01$ for the LA group vs. the No LD group, $P < .01$ for the LH group vs. the No LD group, and $P < .01$ for the LH group vs. the LA group).

Spearman correlation analysis for the entire cohort of patients revealed a significant correlation between leptin and total cholesterol, HDL cholesterol, and insulin levels, and HOMA-IR ($P < .05$; data not shown). Subgroup analysis for the No LD group revealed correlations between leptin and both the insulin level and HOMA-IR ($P < .05$ for each analysis). There were no significant bivariate correlations for subjects in the LA, LD, or MLD groups.

Multiple linear regression analysis evaluating the relationship between leptin and the measured metabolic variables, with controlling for age, sex, total exercise, total NRTI use, and the $CD4^+$ T cell count at diagnosis of HIV infection, revealed independent associations of leptin with the insulin level and HOMA-IR for subjects in the LA group ($P < .01$), and with total cholesterol and triglyceride levels for subjects in the LH group ($P < .05$; table 2). Total exercise performed by the study participants was evaluated using 3 multiple-choice questions, with the type of exercise coded as 1 (walking on level ground and/or swimming), 2 (running, participation in aerobic classes, and/or use of cardiovascular conditioning machine, such as a treadmill or stationary bike), or 3 (weight training), and with intensity coded as 1 (slight exercise), 2 (moderate exercise), or 3 (heavy exercise). Exercise frequency ranged from 0–7 or more sessions per week, and the duration of exercise was classified as either <15 min, 15–29 min, 30–59 min, 60–89 min, or >90 min per session. Cumulative indexes for either aerobic or total (i.e., aerobic and/or resistance) exercise were calculated using the following formula: intensity scale × duration in minutes × number of sessions per week.

A similar multiple linear regression model was constructed, with adjustment for all of the aforementioned variables as well as for total PI use, lower limb fat (as determined by DEXA), and visceral fat (as determined by CT)—3 additional variables that were added because of their potential relationship to insulin resistance. Addition of these variables to the model did not change the results for the LA group; leptin remained associated with both insulin ($\beta$ effect ± SE, 35.981 ± 5.679; $P < .001$) and HOMA-IR ($\beta$ effect ± SE, 6.596 ± 1.404; $P = .001$) in the LA group (data not shown). In the LH group, leptin did not remain associated with total cholesterol or triglycerides, and, in the No LD group, there was a new association between leptin and insulin that was not seen in the original model ($\beta$ effect ± SE, 1.159 ± 0.562; $P < .05$).

Logistic regression analysis is shown in table 3. After controlling for age, sex, total exercise, total NRTI use, and $CD4^+$ T cell count at diagnosis of HIV infection was done, leptin was independently associated with lipoatrophy (adjusted OR, 0.445;

**Table 2. Multiple linear regression analysis of leptin as a predictor of the metabolic variables controlling for age, sex, total exercise, total nucleoside reverse-transcriptase inhibitor (NRTI) use, and CD4+ T cell count at diagnosis of HIV infection.**

| | No LD group | | LA group | | MLD group | | LH group | |
|---|---|---|---|---|---|---|---|---|
| Variable | $\beta$ Effect ± SE | *P* | $\beta$ Effect ± SE | *P* | $\beta$ Effect ± SE | *P* | $\beta$ Effect ± SE | *P* |
| Cholesterol level | | | | | | | | |
| Total | −0.717 ± 4.663 | .88 | 10.177 ± 13.636 | .47 | −4.973 ± 5.679 | .39 | 4.818 ± 1.960 | .04 |
| HDL | 1.026 ± 0.901 | .26 | −1.396 ± 2.153 | .53 | 0.449 ± 0.637 | .49 | −0.249 ± 0.640 | .71 |
| LDL | 1.407 ± 3.552 | .69 | −0.884 ± 9.619 | .93 | −0.214 ± 3.525 | .95 | 2.292 ± 1.502 | .17 |
| Triglyceride level | −14.502 ± 13.772 | .30 | 61.458 ± 60.192 | .32 | −42.015 ± 39.147 | .30 | 13.663 ± 5.512 | .04 |
| Glucose level | −0.109 ± 0.662 | .87 | −2.496 ± 4.225 | .56 | −4.505 ± 2.771 | .08 | 0.516 ± 2.157 | .82 |
| Insulin level | 0.985 ± 0.481 | .05 | 21.676 ± 5.438 | .001 | 1.640 ± 2.011 | .42 | −1.053 ± 1.487 | .50 |
| HOMA-IR[a] | 0.191 ± 0.100 | .06 | 3.90 ± 1.192 | .005 | 0.106 ± 0.798 | .90 | −0.524 ± 0.550 | .37 |

**NOTE.** HDL, high-density lipoprotein; HOMA-IR, insulin resistance determined using the homeostasis model assessment index; LA group, patients with lipoatrophy; LDL, low-density lipoprotein; LH group, patients with lipohypertrophy; MLD group, patients with mixed lipodystrophy (peripheral lipoatrophy with central fat accumulation); No LD group, patients with no evidence of lipodystrophy.

[a] HOMA-IR formula: (fasting insulin level in microunits per milliliter × glucose level in millimoles per liter)/22.5.

95% CI, 0.231–0.856; $P = .02$) and lipohypertrophy (adjusted OR, 1.787; 95% CI, 1.249–2.557; $P < .01$), but not with mixed lipodystrophy (adjusted OR, 1.156; 95% CI, 0.931–1.435; $P = .19$).

## DISCUSSION

The HIV-related lipodystrophy syndrome is increasingly recognized. However, there are many questions that remain unanswered with regard to the syndrome itself and the metabolic abnormalities with which it is associated, specifically the relationship between leptin, insulin resistance, and fat redistribution.

In the present observational study, we demonstrated that there are significant associations between leptin levels and insulin resistance in patients with distinct HIV lipodystrophy phenotypes. We carefully classified patients with regard to the presence of lipoatrophy, lipohypertrophy, or both, and we then evaluated the relationship between leptin and metabolic abnormalities seen in association with the HIV-1 lipodystrophy syndrome. Patients with lipoatrophy have the lowest leptin levels, patients with lipohypertrophy have the highest levels, and patients with either mixed lipodystrophy or no lipodystrophy have intermediate levels. Previous studies evaluating leptin levels in patients with HIV-related lipodystrophy have reported conflicting results, with some studies suggesting a relationship between leptin and lipodystrophy, and with other studies demonstrating no relationship [1, 25–27]. Our results are in agreement with those of Estrada et al. [33], who recently demonstrated that fat loss in the extremities of HIV-1–infected men was associated with low levels of leptin, increased visceral fat, and metabolic abnormalities. Vigouroux et al. [24] found that HIV-related lipodystrophy was associated with insulin resis-

**Table 3. Logistic regression analysis of leptin as a predictor of lipodystrophy, after controlling for age, sex, total exercise, total nucleoside reverse-transcriptase inhibitor (NRTI) use, and CD4+ T cell count at diagnosis of HIV infection.**

| | LA group vs. No LD group | | MLD group vs. No LD group | | LH group vs. No LD group | |
|---|---|---|---|---|---|---|
| Variable | Adjusted OR (95% CI) | *P* | Adjusted OR (95% CI) | *P* | Adjusted OR (95% CI) | *P* |
| Age | 1.054 (0.965–1.152) | .24 | 1.057 (0.979–1.141) | .16 | 0.897 (0.773–1.041) | .15 |
| Sex | 0.145 (0.002–11.563) | .39 | 1.042 (0.089–12.21) | .97 | 6.819 (0.259–179.6) | .25 |
| Total exercise[a] | 1.000 (0.999–1.001) | .61 | 0.999 (0.998–1.000) | .08 | 0.999 (0.998–1.001) | .24 |
| Total NRTI use[b] | 1.013 (1.001–1.025) | .04 | 1.015 (1.004–1.027) | .01 | 1.014 (0.996–1.032) | .12 |
| CD4+ T cell count[c] | 1.001 (0.999–1.003) | .49 | 0.996 (0.993–0.999) | .02 | 0.995 (0.990–0.999) | .02 |
| Leptin level, ng/mL | 0.445 (0.231–0.856) | .02 | 1.156 (0.931–1.435) | .19 | 1.787 (1.249–2.557) | .002 |

**NOTE.** No LD group, patients with no evidence of lipodystrophy; LA group, patients with lipoatrophy; LH group, patients with lipohypertrophy; MLD group, patients with mixed lipodystrophy (peripheral lipoatrophy with central fat accumulation).

[a] Intensity scale × duration in minutes × number of sessions per week.

[b] Cumulative duration (in months) of use of all NRTIs.

[c] At the time of diagnosis of HIV infection.

tance, overt diabetes mellitus, and an elevated triglyceride level, but this group found no difference in leptin levels between patients with or without HIV-related lipodystrophy. Christeff et al. [34] did not find a correlation between leptin and lipodystrophy, but they found elevated levels of leptin overall. It is possible that the conflicting results of these studies are the result of the inclusion of all patients with lipodystrophy, without further stratification by pattern of lipodystrophy. If patients with both lipoatrophy and lipohypertrophy had been analyzed together in one group, the relationships between leptin, lipoatrophy, lipohypertrophy, and insulin resistance that we demonstrated in this study might not have been apparent.

Leptin is produced by adipocytes, and low levels are a consequence of low fat stores in a patient. Thus, the leptin deficiency that we report in patients with HIV-related lipoatrophy in the present study may be a result of fat loss, with decreased synthesis and release of leptin from adipocytes. In contrast, the elevated leptin levels seen in patients with HIV-related lipohypertrophy may signify a state of leptin resistance or overproduction by increased adipose tissue mass. The insulin resistance seen in patients with the lipohypertrophy and mixed lipodystrophy phenotypes in our study was not independently associated with leptin levels, but the relationship between leptin-binding protein or free leptin and insulin resistance in individuals with HIV-related lipohypertrophy is unclear and needs to be studied further.

We have demonstrated that there is a relationship between leptin levels and insulin resistance in individuals with HIV-related lipoatrophy, independent of either central or peripheral fat mass. It is possible that abnormal leptin levels may develop as a consequence of fat wasting in this subgroup of patients, and that hypoleptinemia may subsequently contribute to the metabolic abnormalities seen in association with HIV-related lipoatrophy. Ongoing interventional studies that involve administration of leptin to leptin-deficient subjects with HIV-related lipoatrophy are expected to prove whether the leptin deficiency is causally related to the metabolic abnormalities associated with lipoatrophy. The beneficial effect of leptin replacement on the metabolic parameters in adult women with congenital and acquired lipodystrophy and hypoleptinemia has recently been demonstrated in a small study [14], and it is reasonable to consider, given the many similarities between the syndromes, that leptin replacement may also prove to be of benefit in hypoleptinemic patients with HIV-related lipoatrophy and insulin resistance. Additional prospective studies that involve larger groups of patients who manifest the different lipodystrophy phenotypes must be done to confirm our findings and to further elucidate the complex relationship between leptin and the HIV-associated lipodystrophy syndrome.

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