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ORIGINAL ARTICLE VIROLOGY

# HIV/antiretroviral therapy–related lipodystrophy syndrome (HALS) is associated with higher RBP4 and lower omentin in plasma

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## Abstract

Very little information is available on the involvement of newly characterized adipokines in human immunodeficiency virus (HIV)/antiretroviral therapy (ART)-associated lipodystrophy syndrome (HALS). Our aim was to determine whether apelin, apelin receptor, omentin, RBP4, vaspin and visfatin genetic variants and plasma levels are associated with HALS. We performed a cross-sectional multicentre study that involved 558 HIV type 1–infected patients treated with a stable highly active ART regimen, 240 of which had overt HALS and 318 who did not have HALS. Epidemiologic and clinical variables were determined. Polymorphisms in the *apelin, omentin, RBP4, vaspin* and *visfatin* genes were assessed by genotyping. Plasma apelin, apelin receptor, omentin, RBP4, vaspin and visfatin levels were determined by enzyme-linked immunosorbent assay in 163 patients (81 with HALS and 82 without HALS) from whom stored plasma samples were available. Student's *t* test, one-way ANOVA, chi-square test, Pearson and Spearman correlations and linear regression analysis were used for statistical analyses. There were no associations between the different polymorphisms assessed and the HALS phenotype. Circulating RBP4 was significantly higher ($p < 0.001$) and plasma omentin was significantly lower (p 0.001) in patients with HALS compared to those without HALS; differences in plasma levels of the remaining adipokines were nonsignificant between groups. Circulating RBP4 concentration was predicted independently by the presence of HALS. Apelin and apelin receptor levels were independently predicted by body mass index. Visfatin concentration was predicted independently by the presence of acquired immunodeficiency syndrome. HALS is associated with higher RBP4 and lower omentin in plasma. These two adipokines, particularly RBP4, may be a link between HIV/ART and fat redistribution syndromes.
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## Introduction

Adipose tissue is thought to be an endocrine organ that secrets several adipokines having autocrine, paracrine and endocrine effects. Since the discovery and characterization of leptin in 1994 [1], different adipokines have been involved in the


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development of obesity-associated metabolic comorbidities, such as insulin resistance and dyslipidemia. In the human immunodeficiency virus (HIV) setting, *in vitro* and *in vivo* studies have provided evidence for abnormal cytokine and adipokine synthesis in individuals with HIV/antiretroviral therapy (ART)-associated lipodystrophy syndrome (HALS), particularly of tumor necrosis factor alpha, some interleukins, leptin and adiponectin, among others [2–4]. Our group has studied the relationship between HALS and its associated metabolic derangements and several adipokines, among them resistin [5], fatty acid binding protein [6], zinc-associated glycoprotein [7], leptin and adiponectin [8]. Recent information indicates, however, that adipose tissue produces many more adipokines than those mentioned above, such as apelin, omentin, retinol acid binding protein 4 (RBP4), vaspin and visfatin [9]. Most of these newly characterized adipokines have been linked to insulin resistance in uninfected subjects, and information with respect to their involvement in HALS is scarce [10].

To gain further insight into the role of these peptides with hormone properties in HALS and its associated metabolic derangements, we conducted the present study with a cohort of Spanish white HIV-infected patients receiving ART with and without HALS. Our objective was to assess the association of apelin, apelin receptor, omentin, RBP4, vaspin and visfatin single nucleotide polymorphisms and their circulating levels with HALS and its metabolic complications. Additionally, given the close relationship between adipose tissue and the immune system [11,12], the link between these adipokines and several immunovirologic variables was assessed.

## Patients and methods

### Design, setting and participants

This was a multicentre cross-sectional case–control study which included 558 adult HIV type 1 (HIV-1)–infected patients receiving ART, 318 without HALS and 240 with overt HALS. The sample size was designed to find differences of 13% in the distribution of polymorphisms between groups; assuming a proportion of 40% in the non-HALS risk category, a risk alpha of 5% and a power of 80%, the number of individuals to be included was 240 per group. These numbers have been useful and reproducible for gene analyses of HIV-infected subjects [13]. Patients were recruited consecutively between 2004 and 2006 at the HIV outpatient clinic of the participating hospitals. Patients were selected from among those who were receiving ART, defined as the combination of two nucleoside reverse transcriptase inhibitors plus either a nonnucleoside reverse transcriptase inhibitor or protease inhibitor or inhibitors. All the selected patients had to fulfil the following inclusion criteria: age over 18 years, presence of HIV-1 infection, stable ART regimen for at least 1 year and presence or absence of HALS according to previously defined standardized criteria. Exclusion criteria were the presence of active opportunistic infections, current inflammatory diseases or conditions, consumption of drugs with known metabolic effects such as steroids (systemic, inhaled or topical), antidiabetic or hypolipidaemic drugs and hormones, and plasma C-reactive protein >1 mg/dL. Gene studies of *Apelin, omentin, RBP4, vaspin* and *visfatin* were carried out on all the 558 patients recruited, while circulating apelin, apelin receptor, omentin, RBP4, vaspin and visfatin levels were assessed in a subset of 163 patients (82 without HALS and 81 with HALS) from whom stored plasma samples, drawn when enrolled, were available. Ethics committees from the participating institutions approved the project. Informed consent was obtained from each participant.

### Assessment of HALS

All patients were given a full physical examination to assess the type (lipoatrophy, lipohypertrophy or mixed) and degree (slight, moderate or severe) of lipodystrophy. Criteria for lipoatrophy were one or more of the following: loss of fat from the face, arms and legs; prominent veins in the arms and legs; and a thin bottom. Lipohypertrophy was defined by the presence of one or more of the following criteria: increase in abdominal perimeter, breast and/or neck fat deposition. We defined mixed lipodystrophy as at least one characteristic of lipoatrophy and one of lipohypertrophy concomitantly present in a given patient. Lipodystrophy was categorized in accordance with a scale previously validated [14]: nil (0), slight (1), moderate (2) and severe (3). Doubtful cases were excluded. This categorization was evaluated in the face, arms, legs, buttocks, abdomen, neck and breasts. The sum of the values corresponding to each body area indicated the degree of lipodystrophy: nil (0), slight (1–6), moderate (7–12) and severe (13–18) [5–8,14,15]. In this study we included only extreme lipodystrophy phenotypes (nil vs. severe cases) in order to avoid superposition between groups. To objectively assess the distribution of visceral adipose tissue and subcutaneous adipose tissue, a single-slice computed tomographic scan was performed at the level of L4 in the 558 patients included in this study. The surface of adipose tissue was measured in square centimeters.

### Laboratory methods

*Collection of blood samples.* Blood was drawn from a peripheral vein after overnight fasting. Whole blood was used to determine $CD4^+$ T cell count and for DNA isolation. Plasma and serum were obtained by centrifugation and were stored at −80°C until used.

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*HIV-1 infection-related parameters.* HIV-1 infection was diagnosed by a positive enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot analysis. Plasma HIV-1 virus load was determined by the Cobas Amplicor HIV-1 Monitor Test, v1.5 (Roche Diagnostics, Barcelona, Spain). $CD4^+$ T cell count was analysed in a flow cytometer FAC Scan (Becton Dickinson, San Jose, CA, USA).

*Blood chemistry.* Concentrations of glucose, insulin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides were measured using standard enzymatic methods. The homeostasis model assessment-estimated insulin resistance (HOMA-IR) method (insulin (μIU/ml) × glucose (mmol/L)/22.5) was used to calculate insulin resistance.

*Assessment of polymorphisms.* All the single nucleotide polymorphisms were validated by genotyping methods (KBioscience, Herts, UK). The variations studied were the following: *Apelin,* rs3761581 (G>T) and rs2235306 (C>T); *Omentin,* rs2274910 (C>T); *RBP4,* rs3758538 (A>C), rs3758539 (A>G) and rs34571439 (A>C); *Vaspin,* rs2236242 (A>T); and *Visfatin,* rs1319501 (C>T), rs4730153 (A>G), rs10487818 (A>T) and rs9770242 (A>C).

*Apelin, apelin receptor, omentin, RBP4, vaspin and visfatin plasma levels.* Plasma omentin and visfatin concentration were determined in duplicate using commercial Omentin-1 human ELISA and Visfatin (NAMPT) human ELISA kits, respectively (BioVendor, Heidelberg, Germany). Human RBP4 DuoSet and Human Serpin A12 DuoSet kits (R&D Systems, Minneapolis, MN, USA) were used to analyse plasma concentration of RBP4 and vaspin, respectively. Apelin and its receptor were determined using respective Human Apelin and Human Apelin receptor ELISA kits (Qayee-Bio, Shanghai, China). For each adipokine, intra- and interassay variation coefficients were, respectively: apelin, 5.4% and 5.5%; apelin receptor, 5.8% and 6%; omentin, 4.1% and 4.8%; RBP4, 5.5% and 6.8%; vaspin, 5.5% and 9.2%; and visfatin, 8.7% and 9.5%.

### Statistical analyses

The statistical studies were carried out by SPSS software for Windows, version 17.0 (IBM, Armonk, NY, USA). Firstly, normal distribution and homogeneity of the variances were assessed. Normally distributed data was expressed as mean ± SD, whereas variables with a skew distribution were represented as the median (25th percentile–75th percentile) or transformed using the logarithm function. Categorical variables were expressed as number (percentage). Qualitative variables were analysed by the chi-square test or Fisher's exact test when necessary. Student's *t* test and one-way ANOVA with Bonferroni *post hoc* test were used to compare continuous variables between two groups and more than two groups, respectively. To compare variables that did not fit a Gaussian distribution, we used the Mann-Whitney *U* and Kruskal-Wallis tests in the same way. Associations between quantitative variables were evaluated by Pearson correlation analysis or Spearman correlation for nonnormally distributed variables. The independence of the observed associations was evaluated by linear regression analysis. Differences with a p value of <0.05 were considered significant for all statistical tests.

## Results

### Characteristics of study population

Table I summarizes the demographic, clinical, immunovirologic and treatment characteristics of the 558 patients, who were separated by the presence or absence of HALS. All patients from the HALS group had extreme lipodystrophy phenotype, which included marked peripheral lipoatrophy severe enough to be treated with facial implants. Concomitant central

**TABLE I. Demographic and clinical characteristics of patients categorized according to presence or absence of HALS**

| Characteristic | No HALS ($n$ = 318) | HALS ($n$ = 240) | p |
|---|---|---|---|
| Age (years) | 43.5 ± 9.5 | 45.5 ± 9.7 | 0.01 |
| Male sex, *n* (%) | 227 (71.4) | 171 (71.3) | 1 |
| BMI (kg/m$^2$) | 22.9 ± 3.1 | 23.6 ± 2.8 | 0.01 |
| Waist–hip circumference ratio | 0.89 ± 0.09 | 0.93 ± 0.08 | 0.04 |
| Subcutaneous abdominal fat tissue (cm$^2$)[a] | 128.9 ± 61.3 | 50 ± 30.7 | 0.004 |
| Visceral abdominal fat tissue (cm$^2$)[a] | 37.2 ± 27 | 110.2 ± 68 | 0.01 |
| HCV infection, *n* (%) | 120 (37.7) | 79 (32.9) | 0.23 |
| HIV-1 risk group, *n* (%) | | | |
| Homosexual | 111 (34.9) | 76 (31.7) | 0.4 |
| Heterosexual | 93 (29.2) | 81 (33.8) | 0.35 |
| Injection drug user | 114 (35.8) | 77 (32.1) | 0.35 |
| Other/unknown | 0 | 6 (2.5) | 0.004 |
| Duration of HIV infection (years) | 11.7 ± 5.7 | 13.7 ± 5.9 | 0.002 |
| AIDS, *n* (%) | 85 (26.7) | 118 (49.1) | <0.001 |
| Current $CD4^+$ T cell count (cells/mL) | 474 ± 297 | 560 ± 308 | 0.04 |
| Current log plasma HIV-1 load (copies/mL) | 2.6 ± 1.2 | 2.2 ± 0.8 | <0.001 |
| Current plasma HIV-1 load <200 copies/mL, *n* (%) | 243 (76.4) | 195 (81.3) | 0.17 |
| Exposure to NRTI before ART, yes, *n* (%) | 226 (71.1) | 198 (82.5) | 0.002 |
| Duration of ART (months) | 81 (37–117) | 66 (44–126) | 0.9 |
| NRTI consumption, yes, *n* (%) | 300 (94.3) | 239 (99.6) | <0.001 |
| Cumulative time on NRTI (months) | 128 (72–168) | 159 (98–180) | 0.05 |
| NNRTI consumption, yes, *n* (%) | 220 (69.2) | 168 (70) | 0.8 |
| Cumulative time on NNRTI (months) | 21 (11–36) | 26 (15–54) | 0.008 |
| PI consumption, yes, *n* (%) | 251 (78.9) | 217 (90.4) | <0.001 |
| Cumulative time on PI (months) | 47 (27–88) | 55 (32–84) | 0.15 |
| AZT consumption, yes, *n* (%) | 225 (70.8) | 186 (77.5) | 0.03 |
| Cumulative time on AZT (months) | 45 (24–80) | 41 (10–61) | 0.03 |
| d4T consumption, yes, *n* (%) | 156 (49.1) | 196 (81.7) | <0.001 |
| Cumulative time on d4T (months) | 37 (19–72) | 39 (24–60) | 0.93 |

Data are expressed as mean ± standard deviation or as median and IQR 25–75%. Qualitative variables are expressed as percentages.
ART, antiretroviral therapy; AZT, zidovudine; BMI, body mass index; d4T, stavudine; HALS, human immunodeficiency virus type 1/ART-related lipodystrophy syndrome; HCV, hepatitis C virus; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a]Assessed by computed tomographic scan at level of L4.

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lipohypertrophy was present in approximately 50% of patients. Table 2 lists the metabolic parameters of the subset of 163 patients for whom plasma adipokine measurements were taken. No differences were observed between this subset of patients and the remaining 395 patients from whom plasma samples were not available; the subset is thus representative of the whole cohort.

### Genetic association study

Table 3 lists genetic association study data. All genotypes and allele frequencies studied were distributed according to the predicted Hardy-Weinberg equilibrium, except for *apelin* rs3761581G/T, *apelin* rs2235306C/T and *visfatin* rs10487818G/T, which did not follow this distribution. None of the genetic variants analysed was associated with HALS. Of note, plasma omentin levels were associated with *omentin* rs2274910 polymorphism in patients with HALS, with carriers of the TT genotype having lower plasma omentin levels than carriers of the CC genotype (p 0.009). Otherwise, in patients without HALS, plasma visfatin levels were significantly lower in carriers of the *visfatin* rs1319501 TT genotype than in carriers of the CC genotype (p 0.004). No other polymorphisms in the adipokine genes assessed appeared to influence plasma levels of the adipokines for which they respectively encode.

### Plasma levels of adipokines

*HALS study.* Fig. 1 shows the concentration of circulating apelin, apelin receptor, omentin, RBP4, vaspin and visfatin in patients categorized according to the presence or absence of lipodystrophy. Patients with HALS had significantly lower plasma omentin concentration (p 0.001) and significantly higher plasma RBP4 concentration (p < 0.001) compared to patients without HALS. Patients with pure lipoatrophy had nonsignificantly different circulating adipokine levels than patients with mixed lipodystrophy.

*Relationship between adipokines.* We assessed the correlations between plasma levels of the six adipokines determined. Each correlation was assessed in the whole cohort and in the subsets separately. In the whole cohort, omentin correlated with vaspin (r = 0.26, p < 0.001) and with RBP4 (r = −0.16, p 0.04). RBP4 correlated with visfatin (r = −0.23, p < 0.001). In the HALS subset, omentin correlated with visfatin, vaspin and apelin receptor (r = 0.31, r = 0.29 and r = 0.25 respectively, all p < 0.05); visfatin correlated with vaspin (r = 0.44, p < 0.001), vaspin with RBP4 (r = 0.470, p < 0.001) and RBP4 with apelin receptor (r = −0.24, p 0.03). In patients without HALS, there was a correlation between visfatin and RBP4 (r = −0.22, p 0.05).

*Correlations with metabolic parameters.* We assessed the correlations between plasma levels of the six determined adipokines and total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, glycaemia, insulin and HOMA-IR. Each correlation was assessed in the whole cohort and in the subsets separately. For the entire cohort, RBP4 correlated with triglycerides (r = 0.180, p 0.025), apelin

**TABLE 2. Metabolic data of patients categorized according to presence or absence of HALS**

| Characteristic | No HALS ($n$ = 82) | HALS ($n$ = 81) | p |
|---|---|---|---|
| Glucose (mmol/L) | 5.4 (4.8–6.2) | 5.3 (4.7–6.3) | 0.77 |
| Insulin (μU/mL) | 24.7 (17.9–31.4) | 24.2 (14.8–37.8) | 0.76 |
| HOMA-IR | 6.1 (4.4–9) | 5.8 (3.6–9.9) | 0.58 |
| Cholesterol (mmol/L) | 4.8 (4.1–6.2) | 5.2 (4.3–6.4) | 0.21 |
| HDL cholesterol (mmol/L) | 1 (0.9–1.3) | 1 (0.9–1.3) | 0.77 |
| LDL cholesterol (mmol/L) | 3.2 (2.5–4) | 3.4 (2.5–4.2) | 0.51 |
| Triglycerides (mmol/L) | 1.8 (1.2–2.4) | 1.9 (1.2–3.5) | 0.36 |

HALS, human immunodeficiency virus type 1/antiretroviral therapy–related lipodystrophy syndrome; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; LDL, low-density lipoprotein.
Data are expressed as median and IQR 25–75%.

**TABLE 3. Association between polymorphisms of *apelin, omentin, RBP4, vaspin* and *visfatin* genes and presence of HALS**

| Gene | Genotype | No HALS ($n$ = 318) | HALS ($n$ = 240) | p |
|---|---|---|---|---|
| *rs2235306* | *T/T* | 306 (96.2) | 228 (95) | 0.34 |
| *APELIN* | *T/C* | 9 (2.8) | 6 (2.5) | |
| *C/T* | *C/C* | 3 (0.9) | 6 (2.5) | |
| | *Allele T* | 97.5% | 96.5% | 0.37 |
| *rs3761581* | *T/T* | 303 (95.3) | 233 (97.1) | 0.21 |
| *APELIN* | *T/G* | 8 (2.5) | 6 (2.5) | |
| *G/T* | *G/G* | 7 (2.2) | 1 (0.4) | |
| | *Allele T* | 96.4% | 98.3% | 0.06 |
| *rs2274910* | *C/C* | 155 (48.7) | 114 (47.5) | 0.39 |
| *OMENTIN* | *T/C* | 124 (39) | 104 (43.3) | |
| *C/T* | *T/T* | 39 (12.3) | 22 (9.2) | |
| | *Allele C* | 68.4% | 69.4% | 0.78 |
| *rs34571439* | *A/A* | 198 (62.3) | 152 (63.3) | 0.46 |
| *RBP4* | *C/A* | 110 (34.6) | 76 (31.7) | |
| *A/C* | *C/C* | 10 (3.1) | 12 (5) | |
| | *Allele A* | 79.6% | 79.4% | 1 |
| *rs3758538* | *A/A* | 233 (73.3) | 174 (72.5) | 0.89 |
| *RBP4* | *C/A* | 73 (22.9) | 55 (22.9) | |
| *A/C* | *C/C* | 12 (3.8) | 11 (4.6) | |
| | *Allele A* | 84.7% | 83.8% | 0.71 |
| *rs3758539* | *G/G* | 207 (65.1) | 162 (67.5) | 0.06 |
| *RBP4* | *A/G* | 105 (33) | 66 (27.5) | |
| *A/G* | *A/A* | 6 (1.9) | 12 (5) | |
| | *Allele G* | 81.6% | 81.3% | 0.94 |
| *rs2236242* | *A/A* | 123 (38.7) | 97 (40.4) | 0.29 |
| *VASPIN* | *T/A* | 147 (46.2) | 97 (40.4) | |
| *A/T* | *T/T* | 48 (15.1) | 46 (19.1) | |
| | *Allele A* | 61.8% | 60.8% | 0.79 |
| *rs1319501* | *T/T* | 195 (61.3) | 165 (68.8) | 0.12 |
| *VISFATIN* | *T/C* | 108 (34) | 62 (25.8) | |
| *C/T* | *C/C* | 15 (4.7) | 13 (5.4) | |
| | *Allele T* | 78.5% | 81.7% | 0.21 |
| *rs4730153* | *G/G* | 109 (34.3) | 84 (35) | 0.96 |
| *VISFATIN* | *A/G* | 155 (48.7) | 114 (47.5) | |
| *A/G* | *A/A* | 54 (17) | 42 (17.5) | |
| | *Allele G* | 58.7% | 59% | 0.97 |
| *rs9770242* | *A/A* | 197 (61.9) | 163 (67.9) | 0.13 |
| *VISFATIN* | *C/A* | 109 (34.3) | 64 (26.7) | |
| *A/C* | *C/C* | 12 (3.8) | 13 (5.4) | |
| | *Allele A* | 79% | 81.5% | 0.33 |
| *rs10487818* | *A/A* | 309 (97.2) | 234 (97.5) | 0.97 |
| *VISFATIN* | *T/A* | 6 (1.9) | 4 (1.7) | |
| *A/T* | *T/T* | 3 (0.9) | 2 (0.8) | |
| | *Allele A* | 98.3% | 98.5% | 0.81 |

HALS, human immunodeficiency virus type 1/antiretroviral therapy–related lipodystrophy syndrome.
Genotypes are expressed as *n* (%); allele frequencies, %.

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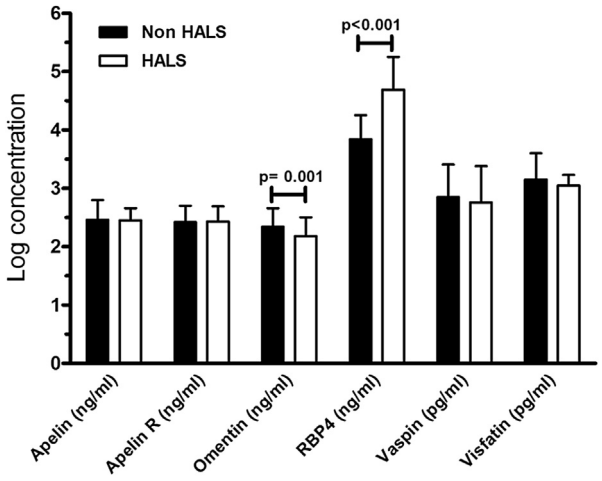


**FIG. 1.** Plasma apelin, apelin receptor, omentin, RBP4, vaspin and visfatin in the subset of 163 patients categorized according to the absence ($n$ = 82) or presence ($n$ = 81) of HALS. Plasma concentration of apelin, apelin receptor, omentin, RBP4, vaspin, and visfatin are expressed as log transformed and as mean ± standard deviation. HALS, human immunodeficiency virus/antiretroviral therapy–related lipodystrophy syndrome.

correlated with HOMA-IR (r = 0.215, p 0.007) and insulin (r = 0.234, p 0.003) and apelin receptor correlated with glucose (r = −0.214, p 0.008). In the HALS subset, the significant correlations observed were between RBP4 and triglycerides (r = 0.246, p 0.032), and between apelin and insulin (r = 0.436, p < 0.001) and HOMA-IR (r = 0.404, p < 0.001). In patients without HALS, there were significant correlations between omentin and glucose (r = 0.238, p 0.033), between vaspin and HOMA-IR (r = 0.222, p 0.048) and between apelin receptor and glucose (r = −0.232, p 0.038).

*Correlations with immunovirologic parameters.* The correlations between circulating adipokines and current plasma HIV virus load and current $CD4^+$ T cell count were evaluated, and each correlation was assessed in the whole cohort and the subsets. In the whole cohort, and as far as current HIV virus load is concerned, omentin and visfatin had a positive correlation (r = 0.193, p 0.02; and r = 0.181, p 0.032, respectively), while RBP4 had a negative correlation (r = −0.220, p 0.008). In the HALS subset, visfatin was significantly correlated with current HIV virus load (r = 0.379, p 0.001). In the non-HALS subset, there was a correlation between apelin receptor and current HIV virus load (r = −0.333, p 0.005) and between omentin and current $CD4^+$ T cell count (r = −0.277, p 0.023).

*Regression analyses.* The associations between circulating adipokine levels and several demographic and clinical variables (age, sex, body mass index (BMI), acquired immunodeficiency syndrome (AIDS), immunovirologic parameters, antiretroviral drugs used, hepatitis C virus (HCV), metabolic parameters) were assessed by Student's $t$ test and Spearman correlation. The significant associations observed in the univariate analysis (data not shown) were further studied with linear regression analyses considering the levels of apelin, apelin receptor omentin, RBP4, vaspin and visfatin as dependent variables. Age, BMI, the above-mentioned bivariate associations and those observed in the univariate analysis of clinical parameters were included as independent variables. For omentin, none of the variables included in the multivariate analysis (age, BMI, HALS, HCV, d4T consumption, current HIV virus load, $CD4^+$ T cell count) predicted circulating levels. Age, BMI, HCV, current HIV virus load and AIDS were the variables included in the regression model for visfatin (R = 0.598). AIDS was useful for predicting visfatin concentration (B = 0.402; p 0.009). Regression model for vaspin was not constructed as a result of its lack of associations with other variables. For RBP4, the model had a multiple correlation coefficient of R = 0.528, and circulating RBP4 concentration was independently predicted by the presence of HALS (B = 0.261; p 0.024). Variables also included in this model were the same as in omentin model plus triglycerides, exposure to nucleoside reverse transcriptase inhibitors before ART and protease inhibitor consumption. As circulating apelin levels are concerned, the model included age, BMI, insulin, HOMA-IR and d4T consumption (R = 0.489), with BMI being the only predictive factor (B = −0.011; p 0.039). Plasma glucose levels, d4T consumption, age and BMI were included in apelin receptor multivariate analysis (R = 0.513). BMI was the only variable independently associated with plasma apelin receptor levels (B = 0.011; p 0.03).

## Discussion

Given that adipose tissue pathology and the prevalence of metabolic derangements in obesity and HALS have some similarities, investigators have suggested that these two conditions share common pathophysiologic and molecular mechanisms [16]. This has been demonstrated consistently with respect to the involvement of some adipokines, such as leptin, adiponectin, resistin and FABP4, among others [5–8]. In the present study, we explored whether perturbations in apelin, apelin receptor, omentin, RBP4, vaspin and visfatin (also known as nontraditional adipokines, given that they have recently been characterized, and their role in obesity and in insulin resistance is only just starting to be explored) do exist in the HALS setting. Our data indicate that genetic variants of these adipokines are not associated with HALS; we found, however, that HALS patients have changes in circulating omentin and RBP4, while the

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remaining adipokines assessed are not perturbed. A question arises as to the reasons that might explain the positive association of plasma adipokines with HALS while genetic variants show no association. It could be speculated that the fat redistribution itself, rather than the effect of genetic variants, is responsible for the changes in circulating omentin and RBP4 in the HALS setting, given the lack of association between HALS and the polymorphisms assessed.

Omentin is secreted mainly in visceral adipose tissue and is down-regulated in obesity and in diabetes [17]. Omentin is regulated by insulin and glucose and has insulin-sensitizing properties [18]. Recent data indicate that circulating omentin levels are lower in nascent metabolic syndrome and that omentin might have beneficial effects in these patients [19]. There is so far no data regarding omentin in the setting of HIV-infected patients. In this study, we observed that patients with HALS had lower circulating levels of omentin compared to those without HALS. This agrees with the finding that patients with obesity [20] and with metabolic syndrome [19] have lower levels of omentin. Surprisingly, however, omentin was not correlated with any of the metabolic variables assessed. Additional interesting findings were the strong positive correlation between omentin and HIV virus load, and the negative correlation between omentin and $CD4^+$ T cell count (in the non-HALS subset only), suggesting an immunovirologic modulatory effect for this adipocytokine; omentin could therefore represent a link between adipose tissue and immune system [11,12] and merits further investigation.

RBP4 is the carrier of circulating retinol. It has been convincingly demonstrated that it contributes to insulin resistance in obesity and in type 2 diabetes mellitus [21,22]. In HIV-infected patients, an Austrian study showed that ART increased circulating RBP4 levels [23], the amount of which was subsequently reduced when rosiglitazone was added [24]. Investigations carried out in South Korea and Taiwan indicate strong association between RBP4 and metabolic syndrome or insulin resistance [25,26], but as far as HALS is concerned, studies in adults and in children have reported no such association [27,28]. Our data indicate that there is a correlation between RBP4 and triglycerides. Retinol is a precursor in the synthesis of the nuclear hormone receptors RXR and RAR [29]. RXR can form heterodimers with subfamily 1 nuclear receptors like PPARs, which regulate the transcription of genes involved in fatty acid metabolism [30]. This could explain the relationship between RBP4 and triglycerides. Furthermore, we found that HALS is associated with higher circulating RBP4 levels; in fact, the presence of HALS was the strongest independent predictor of RBP4 levels. The discrepancy between our results and those reported previously might be due to those studies only having small patient cohorts [27,28], which can yield unreliable data, whereas there are more than 80 patients per arm (with and without HALS) in our cohort.

Apelin is an adipokine expressed in several cells, among them the adipocytes. In uninfected subjects, apelin has been shown to regulate insulin sensitivity [31] and seems to be a promising therapeutic target for type 2 diabetes mellitus [32]. This is in accordance with the strong correlation between apelin and insulin resistance that we found in our patients, particularly in those with HALS. Of note, it has been shown that apelin might play a role in HIV infection, since the apelin receptor may act as a HIV coreceptor *in vitro* [33] and apelin itself inhibits HIV entry into the cell [34]. We have found that in humans infected with HIV, the circulating apelin receptor associates negatively with HIV virus load in patients without HALS, thus emphasizing a potential involvement of this adipokine in some immunovirologic events in patients infected with HIV.

Another recently characterized adipokine is vaspin, also known as serpin. It is predominantly secreted in visceral adipose tissue and exerts insulin-sensitizing actions [35]. Elevated vaspin mRNA expression in adipose tissue and higher plasma vaspin levels have been reported in patients with insulin resistance, type 2 diabetes mellitus and obesity [36]. To our knowledge, the present study is the first to assess vaspin in HIV-infected patients, and the data suggest that this adipokine is not involved in HALS and in metabolic disturbances in the HIV setting.

Visfatin is expressed and regulated mainly in adipose tissue. It has strong insulin-mimetic activity and it has been implicated in insulin resistance, metabolic syndrome, cardiovascular disease and obesity [37,38]. Previous studies on HIV-infected patients have shown that both ART [23] and rosiglitazone [24] increase plasma visfatin levels; however, the relationship between visfatin and metabolic derangements in infected patients is inconsistent [23,39]. In our study, no association between circulating visfatin and insulin resistance or dyslipidemia could be established, thus confirming the findings of Parfieniuk-Kowerda et al. [39]. On the other hand, in the current study, visfatin was positively associated with plasma virus load and with AIDS. Given that *in vitro* studies have shown that visfatin is involved in HIV-Tat transactivating activity [40], and that it is also able to inhibit HIV at an early stage in its life cycle [41], our clinical data reinforce the potential involvement of visfatin in HIV immunopathogenesis.

We acknowledge that the present study has some limitations, among them the cross-sectional nature of our design, which provides associations but not causality. Also, the multiple comparisons performed in this study mean that some of the associations that we found should be considered with caution.

In summary, we found a strong relationship between HALS and omentin and RBP4, and the presence of HALS

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independently predicted circulating RBP4 levels. Omentin, apelin receptor and visfatin may be involved in HIV immunopathogenesis, and this hypothesis merits further investigation.

## Transparency declaration

All authors report no conflicts of interest relevant to this article.

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