Differentially altered molecular signature of visceral adipose tissue in HIV-1-associated lipodystrophy

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ABSTRACT

Objective: Lipodystrophy in HIV-1-infected, antiretroviral-treated-patients is often associated with opposite alterations in adipose tissue depots: lipoatrophy of subcutaneous adipose tissue (SAT) versus lipohypertrophy of visceral adipose tissue (VAT). We determined the specific molecular alterations in VAT relative to SAT in patients.

Design: We analyzed the expression of marker genes of mitochondrial function, adipogenesis and inflammation in a unique collection of eight biopsies of omental VAT from HIV-1-infected, antiretroviral-treated patients with lipodystrophy. For comparison, we analyzed SAT from ten patients, and SAT and VAT from ten non-infected individuals.

Methods: Quantitative real-time PCR of mitochondrial DNA and gene transcripts; immunoblot and multiplex for quantification of specific proteins.

Results: Similar mitochondrial DNA depletion and abnormal increases in mitochondrial protein levels were found in VAT and SAT from patients. Transcript levels of adipogenesis and metabolism marker genes were unaltered in VAT, but were decreased in SAT. TNFα and CD68 were similarly induced in both adipose depots from patients, but other markers of inflammation-related pathways showed distinct alterations: interleukin-18 and IL1RN were induced only in SAT, whereas interleukin-6, −8 and MCP-1 expression was reduced in VAT but not in SAT.

Conclusions: Mitochondrial alterations are similar in VAT and SAT from patients whereas adipogenic gene expression is decreased in SAT but unaltered in VAT, highlighting the relevance of adipogenic processes in the differential alterations of fat depots. Specific disturbances in inflammatory status in VAT relative to SAT are present. Milder induction of pro-inflammatory signaling in VAT could be involved in preventing fat wasting in this depot.
INTRODUCTION

Lipodystrophy syndrome is among the major disturbances occurring in HIV-1-infected patients, with prevalence rates of approximately 40-50%\(^1\). This syndrome is characterized by a complex set of alterations in adipose tissue that mainly involve peripheral lipoatrophy—a severe loss of subcutaneous adipose tissue in the face, arms and legs—often accompanied by visceral lipohypertrophy, an enlargement in visceral adipose tissue from the abdomen and breast (in women). Less prevalent is the occurrence of lipomatosi which usually manifests as an enlargement in the dorso-cervical area (i.e., “buffalo hump”)\(^1,2\). Although these alterations do not necessarily all occur together, the same patient may show lipoatrophy in the face and arms and enlarged adiposity in the visceral area. In addition, HIV-1 patients with lipodystrophy show metabolic alterations reminiscent of the metabolic syndrome, particularly dyslipidemia and insulin resistance. These alterations lead to enhanced cardiovascular risk and favor the development of diabetes. Despite the fact that the trigger of the syndrome is a complex combination of HIV-1-infection and drug treatment-related events, inflammatory processes and lipotoxicity appear to be likely mechanisms for the development of the syndrome\(^3,4\). The recent development of novel antiretroviral drugs with lower toxicity has led to a decrease in the frequency of overt peripheral lipoatrophy. However, visceral lipohypertrophy and associated metabolic alterations remain a common concern in treated patients\(^5\).

In order to understand the etiopathogenesis of lipodystrophy in HIV-1-patients, several studies have analyzed changes in gene expression appearing in adipose tissue of patients. But practically all such studies have been performed using biopsies obtained from subcutaneous fat depots, which is an adipose depot that suffers lipoatrophic alterations. The results of such studies established decreased mitochondrial DNA (mtDNA) content, altered expression of mtDNA-encoded genes, reduced expression of master regulators of adipogenesis and their targets as well as
up-regulation of genes involved in inflammation in adipose tissue from patients showing full-blown lipodystrophy\(^6\)-\(^9\). Furthermore, studies of patients with differences in HIV-1 infection status, antiretroviral treatment, and appearance of lipodystrophy suggested that some alterations in adipose tissue gene expression occur as a consequence of infection whereas other disturbances are more related to treatment or appear only in association with the establishment of full-blown lipodystrophy\(^8\).

Whatever its etiology, lipodystrophy syndrome can be considered a consequence of adipocyte dysfunction. However, opposite clinical changes occur in subcutaneous (lipoatrophy) versus visceral (lipohypertrophy) adipose tissues. To date, no data are available on the specific molecular alterations in visceral fat from HIV-1 patients, a limitation that surely reflects the difficulty of attaining such adipose samples.

Here we analyzed mRNA and protein expression of several markers of mitochondrial function, adipogenesis and inflammation in a unique collection of intra-abdominal visceral (omentumal) adipose tissue (VAT) biopsies from HIV-1-infected, HAART (Highly Active Antiretroviral Therapy)-treated patients with lipodystrophy. This specific marker gene expression profile was compared to that of abdominal subcutaneous adipose tissue (SAT) from patients, and to those of SAT and VAT from non-infected control individuals.

**METHODS**

All patients and controls provided informed written consent to participate in the study. The study was approved by the Ethics Committee of Hospital de la Santa Creu i Sant Pau, Barcelona. Patients with opportunistic infections, neoplasms, or fever of undetermined origin were excluded from the study. At the time of enrollment, no patient or control individuals were using any other drug known to influence glucose metabolism or fat distribution, such as anabolic hormones or systemic corticosteroids, uridine, recombinant human growth hormone or appetite stimulants or suppressants. For
research purposes, biopsy samples of SAT from controls and patients showing lipodystrophy were taken from the abdominal area by needle-aspiration under 1% lidocaine local anesthesia. Samples of omental VAT were obtained on occasion of elective laparoscopic cholecystectomy. No patient had cholecystitis or any other cause of acute abdomen, neither had neoplasia. Tissue samples were immediately frozen in liquid nitrogen. After homogenization in RA1 buffer (Macherey-Nagel, Düren, Germany), an aliquot was used for isolation of DNA, performed using a standard phenol/chloroform extraction methodology. RNA was obtained using an affinity-based column methodology (NucleoSpin, Macherey-Nagel) and included on-column DNA digestion (rDNase; Macherey-Nagel). A 0.5-μg sample of total RNA was transcribed into cDNA using MultiScribe reverse transcriptase and random-hexamer primers (TaqMan Reverse Transcription Reagents; Applied Biosystems, Carlsbad, CA, USA). For quantitative mRNA expression analyses, TaqMan RT-PCR was performed on the ABI-PRISM 7700HT sequence detection system (Applied Biosystems). The TaqMan RT-PCR reaction was performed in a final volume of 25 μl using TaqMan Universal PCR Master Mix, No AmpErase UNG reagent and the specific gene expression primer pair probes (Applied Biosystems).

The Assay-on-demand probes used were as follows: 18S rRNA, Hs99999901_s1; CEBPA (CCAAT/enhancer-binding protein-α), Hs00269972_s1; LPL (lipoprotein lipase), Hs00173425_m1; TNFα (tumor necrosis factor-α), Hs00174128_m1; PPARG (peroxisome proliferator-activated receptor-γ), Hs00234592_m1; adiponectin, Hs00605917_m1; CD68, Hs00154355_m1; SLC2A4/GLUT4 (glucose transporter type-4), Hs00168966_m1; IL (interleukin)-6, Hs00174131_m1; IL-8, Hs00174103_m1; IL-18, Hs99999040_m1; IL1RN (IL-1 receptor antagonist), Hs00893625_m1; CCL2/MCP1 (monocyte chemoattractant protein-1), Hs00234140_m1; CYCS (cytochrome-c), Hs01588973_m1; MT-CYB
(mitochondrial cytochrome-b), Hs02596867_s1; COX4I1 (mitochondrial cytochrome c oxidase subunit IV, isoform 1), Hs00266371_m1; MT-COII (mitochondrial cytochrome c oxidase subunit II), Hs02596865_g1. Quantification of mtDNA was performed using cyt-b prove (MT-CYB) and referred to nuclear DNA, as determined by the amplification of the intronless gene CEBPα (CEBPA). Appropriate controls with no RNA, primers or reverse transcriptase were included in each set of experiments. Each sample was run in duplicate, and the mean value of duplicate was used to calculate the mRNA expression of the gene of interest, which was normalized to that of the reference control (18S rRNA) using the comparative (2\(^{-\Delta\Delta CT}\)) method, following the manufacturer’s instructions. Parallel calculations were performed using PPIA (peptidylprolyl isomerase-A; Hs99999904_m1) and HPRT1 (hypoxanthine phosphoribosyltransferase-1; Hs99999909_m1) as reference genes and yielded essentially the same results.

For quantification of protein levels, adipose tissue samples were homogenized in cold buffer (10 mM HEPES pH 7.5, 5 mM EDTA, 5 mM dithiothreitol, 5 mM MgCl\(_2\)), and a cocktail of protease inhibitors (Complete-mini, Roche, Sant Cugat, Spain). For Western blot analysis, homogenates containing 40 µg of protein were mixed with equal volumes of 2 x sodium dodecyl sulfate (SDS) loading buffer, incubated at 90°C for 5 min, and electrophoresed on SDS/polyacrylamide gels. After transferring to Immobilon-P membranes (Millipore, Billerica, MA, USA), proteins were probed using antibodies directed against sterol regulatory element binding transcription factor-1 (SREBP1, K-10; Santa Cruz Biotechnology, Santa Cruz, CA, USA), β\(_2\)-microglobulin (Dako, Glostrup, Denmark), cytochrome c oxidase subunit IV (COIV; Molecular Probes, Leiden, The Netherlands), and total OXPHOS (MitoSciences, Oregon, USA). Goat anti-mouse and anti-rabbit HRP-conjugated antibodies (Bio-Rad, Hercules, CA, USA/Santa Cruz Biotechnology) and ECL reagents (Immobilon Western; Millipore) were used to detect the immunoreactive signals. Membranes were stained with Coomassie blue
(Sigma-Aldrich, St Louis, MO, USA) to normalize the amount of protein loaded. Multi-Gauge software (Fujifilm) was used for densitometric analyses. MCP-1, IL-6, IL-8, total plasminogen activator inhibitor type-1 (PAI-1), and hepatocyte growth factor (HGF) were quantified in adipose tissue extracts using a multiplex system (Milliplex human adipokine; Linco Research/Millipore, Saint-Charles, MO, USA) and Luminex100ISv2 equipment.

The median and the 25th-75th percentiles (interquartile range) were used to describe non-normally distributed quantitative data; otherwise, means and standard deviations were reported. Where appropriate, analysis of variance or Student's t-tests was used to determine significance of differences between groups. Analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, IL, USA) and the SAS version 9.1.3 software (SAS Institute Inc., Cary, NC, USA). Significance was established at the 0.05 level (two-sided).

RESULTS

Population studied.

Demographics, treatment data, anthropometric and biochemical parameters of patients and controls are shown in Table 1. Control and patient groups were no significantly different in age, gender or BMI. The waist circumference tended to be higher in patients than in controls, but differences did not reach statistical significance. For the two HIV-1-infected groups, no parameter related to cumulative antiretroviral drug treatment, systemic metabolic parameters or viral load indicators differed significantly. However, HIV-1 patients showed significantly higher levels of blood glucose and triglycerides, as well as higher HOMA-IR index values, indicative of insulin resistance, compared to controls in agreement with previous reports\textsuperscript{8,9}. The patient groups showed facial peripheral lipoatrophy, based in objective scales as reported by Fontdevila \textit{et al.}\textsuperscript{10}. 
Mitochondrial-DNA content and expression of marker genes of mitochondrial function in VAT and SAT from HIV-1-infected patients.

The alterations related to adipose tissue mitochondrial toxicity are depicted in Figure 1. There was a significant depletion in mtDNA levels in both subcutaneous and visceral adipose depots from HIV-1-infected patients compared to those from healthy controls (Fig. 1A). Among mtDNA-encoded transcripts, both cytochrome b (cyt b) mRNA and subunit II of cytochrome oxidase (COII) mRNA were significantly reduced only in subcutaneous fat and not in visceral fat from patients (Fig. 1B). However, mRNAs for nuclear-encoded cytochrome c (cyt c) and subunit IV of cytochrome oxidase (COIV) were significantly lower in both SAT and VAT from patients. Furthermore, we analyzed how mitochondrial toxicity translates to alterations in mitochondrial proteins (Fig. 1C). We observed that both types of adipose tissue depots from patients showed increased levels of both nuclear-encoded (ATPsynα) and mtDNA-encoded (COII) mitochondrial proteins. However, these alterations were not common to all mitochondrial proteins analyzed. For instance, complex II FeS protein levels were not significantly different among fat samples from patients compared to those from controls. For COIV protein, a significant increase was observed only in visceral fat from patients. In summary, adipose tissue from patients exhibited profound alterations in mtDNA, transcript and protein levels, in keeping with the overall mitochondrial toxicity often associated with lipodystrophy. The present data establish, however, that for most of the parameters analyzed, alterations in VAT were similar to those in SAT, and only minor differential changes appeared in the omental VAT depot of patients.
Expression of adipogenic genes in VAT and SAT from HIV-1-infected patients.

Consistent with previous reports, the expression of the master gene for adipogenesis, PPARγ, as well as its metabolic targets involved in glucose uptake (Glut4) and fatty acid uptake (LPL) were significantly repressed in subcutaneous fat from patients (Fig. 2A). The same was observed for the adipokine adiponectin. In sharp contrast to the observations in subcutaneous fat, visceral fat exhibited no differences in any of the genes analyzed between patients and controls. We extended this analysis to the study of the SREBP1 protein, another master regulator of adipogenesis for which enhanced protein levels have been reported in subcutaneous fat from HIV-1-patients with lipodystrophy. Effectively, SREBP1 protein levels in SAT were significantly higher in patients than in controls. In contrast, no significant difference was found for SREBP1 protein in VAT from patients (Fig. 2B).

Analysis of the inflammation marker gene profile in VAT and SAT from HIV-1-infected patients.

We observed that SAT from patients showed high levels of the pro-inflammatory cytokine TNFα respect to healthy controls, as previously reported. A similar difference was observed in comparisons of VAT from controls and patients. Parallel alterations were found for transcript levels of CD68, a marker of infiltrating macrophages. Despite these common alterations in subcutaneous and visceral fat from patients, extending this analysis to a more expanded set of marker genes related to inflammatory signaling revealed some differences between visceral and subcutaneous fat from patients. For example, IL-18 was dramatically induced in subcutaneous fat from patients but unaltered in visceral fat. A similar pattern of changes was found for interleukin-1 receptor antagonist (IL1RN) transcript levels. Conversely, IL-6, IL-8 and MCP-1 transcript levels were unaltered in subcutaneous fat from patients but were
significantly down-regulated in visceral fat. To gain insight into differential inflammation-related alterations in VAT from patients, we analyzed changes in several representative proteins. First, we found that protein levels of β2-microglobulin, which is a target gene of TNFα, were similarly increased in subcutaneous and visceral fat from patients. In contrast, IL-6, IL-8 and MCP-1 protein levels showed totally distinct alterations in the two adipose depots. In SAT from patients, the levels of these cytokines were either unchanged or even mildly induced (MCP-1). Conversely, IL-6, IL-8 and MCP-1 levels were dramatically reduced only in VAT from patients. This pattern of alterations was not common to all inflammation-related proteins. For instance, the levels of PAI-1, a natural inhibitor of fibrinolysis produced by adipose tissue (among other tissues) that increases in the plasma of patients with HIV-1/HAART-associated lipodystrophy, were similarly reduced in both SAT and VAT from patients. The levels of HGF, an angiogenic factor known to be released by adipose tissue under pro-inflammatory conditions, were not significantly altered in any adipose tissue depot from patients relative to controls.

**DISCUSSION**

In the present study, we provide the first comparative characterization of the alterations in the molecular signature of VAT relative to SAT in HAART-treated, HIV-1-infected patients with lipodystrophy.

Mitochondrial toxicity is claimed to be a relevant contributor to certain features of lipodystrophy, especially peripheral lipodystrophy. Collectively, the present data indicate that most of the alterations indicative of mitochondrial toxicity occur similarly in visceral and subcutaneous fat of patients, despite the opposite gross modifications in HIV-1-lipodystrophy (lipohypertrophy versus lipodystrophy in VAT and SAT, respectively). As a general trend, all alterations commonly found in SAT from patients—mtDNA
depletion, lower expression of transcripts for some mitochondrial proteins, and a reactive increase in mitochondrial protein levels \(^{7,8,15-17}\)—appeared to occur similarly in VAT. These findings suggest that mitochondrial alterations are unlikely to determine the opposite behavior of the two fat depots in response to viral- and drug-induced insults. However, the possibility that VAT is less sensitive to a given extent of mtDNA depletion than SAT cannot be excluded. It has been reported that healthy human VAT is equipped with more mitochondria and more abundant mtDNA than SAT\(^{18}\), a trend that was also observed in our study. In fact, progressive replacement of drugs with high mitochondrial toxicity (e.g. thymidine analogs) with less mitochondrial-damaging drugs in treatment regimens ameliorates signs of subcutaneous fat atrophy, but not visceral hypertrophy or induction of systemic metabolic syndrome\(^{4,17,19}\).

In contrast, the pattern of alterations in the expression of adipogenesis-related genes, including those encoding molecular controllers of cellular adipogenesis (PPAR\(_\gamma\), SREBP1), as well as transporters (GLUT4) and enzymes (LPL) involved in metabolic accretion processes, was dramatically different in VAT and SAT from patients. A profound repression in the expression of these genes as well as abnormal induction of unprocessed SREBP1 protein has been reported in subcutaneous fat from patients\(^{6,20}\), a finding confirmed in the present study. However, VAT was totally refractory to these alterations. This is consistent with the lack of atrophy of this depot and highlights the relevance of adipogenic differentiation processes in eliciting the differential alterations of fat depots in patients.

Previous studies have identified local induction of inflammation in SAT from HIV-1 patients\(^{6,21}\). Our present findings indicate that VAT and SAT from patients share alterations indicative of a pro-inflammatory environment (i.e. similar induction of TNF\(\alpha\) expression, indications of parallel induction of macrophage recruitment). However, there are several differences in the alterations of specific subsets of inflammation-
related pathways, strongly suggesting a differential inflammatory response to the HIV-1-infection/HAART treatment-related insults in the two adipose depots. In VAT from patients, the IL-18 induction found in SAT did not occur. Moreover, expression of the prototypical pro-inflammatory cytokine proteins IL-8 and MCP-1, which was either unchanged (IL-8) or increased (MCP-1) in SAT from patients relative to controls, was reduced in VAT from patients. IL-6 was found to be unaltered in SAT from patients, similar to some previous reports but in contrast with other studies. The reasons for such discrepancies are unclear, although the use of different techniques to recover abdominal SAT might affect the degree to which IL-6 gene expression is altered. Consistent with this possibility, our results were similar to those obtained by other studies that also used needle-aspiration, but different from those that used surgical biopsy or liposuction. Additionally, it is generally reported that other cytokines, such as TNFα, are induced in SAT from HAART-treated, HIV-1-infected patients with lipodystrophy, often to a greater extent and with stronger statistical significance than IL-6 induction. Accordingly, significant negative correlations with adipogenic markers have been reported for TNFα, but not IL-6, mRNA expression in SAT from patients. In any case, present analysis showed that IL-6 expression in VAT from patients was even reduced relative to controls. These observations lead to the intriguing conclusion that several features of the pro-inflammatory reaction in HIV-1 patients are moderated, and even impaired, in VAT compared with SAT. In this sense, ex-vivo studies have previously suggested a greater sensitivity of SAT than VAT to inflammation induced by protease inhibitor drugs.

The preferential induction of anti-inflammatory cytokines such as IL1RN in SAT could also be a reaction to the enhanced exposure to pro-inflammatory cytokines in this adipose depot, similar to what has been reported in other conditions of pro-inflammatory insults in fat, such type II diabetes and obesity. Moreover, the
induction of IL1RN expression in SAT from patients is consistent with previous reports of the simultaneous recruitment of M2-type macrophages (reparative, anti-inflammatory) and M1-type macrophages (pro-inflammatory) in this adipose depot.

Studies on healthy human adipose depots have indicated that the basal level of pro-inflammatory signaling is higher in VAT, possibly due to the presence of greater numbers of resident immune cells (macrophages, lymphocytes). Although the limitations of the present study (low number of patients, samples from SAT and VAT corresponding to different patients) do not allow definitive conclusions, we observe a trend toward higher expression of several pro-inflammatory genes (IL-6, IL-8, MCP-1) in VAT relative to SAT in healthy individuals. It is possible that such a higher underlying pro-inflammatory environment in healthy VAT results in a differential inflammatory response to HIV-1/HAART insults. Further research that is unencumbered by the intrinsic difficulties and limitations associated with this first study of VAT from patients will be needed to clarify this point.

By now, it is tempting to speculate that systematic induction of most pro-inflammatory cytokines in SAT is associated with wasting processes that lead to atrophy, whereas milder pro-inflammatory induction in VAT protects against wasting. In fact, if the initial induction of intense local inflammatory signaling is responsible for the impairment in adipogenic processes in lipoatrophic subcutaneous fat (e.g., via repression of PPARγ by pro-inflammatory cytokines), as has often been proposed, a milder inflammation in VAT could account for preservation of visceral adipogenic processes. On the other hand, it cannot be discounted that lipoatrophy itself enhances the induction of pro-inflammatory pathways in SAT, whereas in VAT, the lack of atrophy prevents from some features of enhanced inflammation occurring in the subcutaneous depot. The fact that the dramatic induction of IL-18, a cytokine highly involved in apoptotic processes, is only observed in the subcutaneous fat of patients...
is consistent with the notion that some of the pro-inflammatory signaling induction in SAT may be directly associated with the ongoing lipoatrophic process.

Several previous studies have compared the atrophic versus hypertrophic alterations in SAT from patients by the analyzing the dorso-cervical enlarged fat pad (“buffalo hump”) that develops in some HIV-1 patients\textsuperscript{20,35}. It is worth noting that, despite this dorsal fat depot is not visceral fat, its enlargement is associated with a depletion of mtDNA similar to that observed in lipoatrophic subcutaneous fat from patients, but preserved expression of adipogenic genes and absence of induction of pro-inflammatory signaling. These observations are consistent with present findings in the sense that overt inflammation appears to be closely associated with a lipoatrophic status in adipose tissue from HIV-1 patients, regardless of anatomical location.

Several limitations affect the extent to which conclusions can be drawn from the present study. The low number of samples, especially the limited availability of omental VAT from HIV-1 patients, limits the capacity to unequivocally clarify issues such as the role of drug-treatment patterns or the actual significance of gene expression data when differences between groups lack statistical significance. Some heterogeneity in individual composition (older and more frequently female patients in the VAT control group) can also be considered a limitation. Moreover, the lack of availability of biopsies of VAT and SAT from the same individuals also precludes a proper assessment of the impact of individual variability on gene expression patterns in adipose tissue. Finally, the present study did not seek to directly determine the cellular origin of adipose tissue alterations in the expression profiles of cytokine genes, most of which, in addition to being expressed by adipocytes, are intensely expressed and released by infiltrating macrophages and even T-lymphocytes in adipose depots. Despite these limitations, the present results constitute the first systematic analysis of gene expression in VAT from HIV-1-infected patients on HAART and reveal marked differences in the pattern of alterations in visceral fat relative to those in subcutaneous fat of patients with
lipodystrophy. Given the prominent role of VAT in the control of systemic metabolic alterations, understanding the alterations in this fat depot in patients may aid in the development of metabolic-friendly therapies and prevention strategies in the treatment of the HIV-1 patient.
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FIGURE LEGENDS

Figure 1
Mitochondrial DNA abundance and expression of mitochondrial function marker genes in subcutaneous (SAT) and visceral (VAT) adipose tissues from HIV-1-infected patients with lipodystrophy (LD), compared with SAT and VAT from healthy controls (C).
(A) mtDNA content (mean ± SEM) expressed as a ratio to nuclear DNA (in arbitrary units). (B) Quantification of mRNAs for specific mitochondrial marker genes, normalized to the levels of 18S rRNA (endogenous control), expressed as means ± SEMs. (C) Representative immunoblot images and densitometric quantitation of protein bands, expressed as means ± SEM for each group (in arbitrary units). *P<0.05, patients vs. controls.

Figure 2
Expression of adipogenic marker genes in SAT and VAT from HIV-1-infected patients with lipodystrophy (LD), compared with that in SAT and VAT from healthy controls (C).
(A) Quantification of mRNAs for specific adipogenic marker genes, normalized to the levels of 18S rRNA (endogenous control), expressed as means ± SEMs. (B) Representative immunoblot images and densitometric quantitation of protein bands, expressed as means ± SEM for each group (in arbitrary units). *P<0.05, patients vs. controls.
Figure 3
Expression of inflammation marker genes in SAT and VAT from HIV-1-infected patients with lipodystrophy (LD) compared with those in SAT and VAT from healthy controls (C).

(A) Quantification of mRNAs for specific inflammation marker genes, normalized to the levels of 18S rRNA (endogenous control), expressed as means ± SEMs. (B) Protein histograms show means ± SEM for each sample group. Upper panel: Representative image of β2-microglobulin. *P<0.05, patients vs. controls.
Table 1. Demographics, treatment data, anthropometric and biochemical parameters in patients and controls.

<table>
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<tr>
<th>Parameters</th>
<th>CONTROL: Subcutaneous (n=10)</th>
<th>Visceral (n=10)</th>
<th>HIV-infected-HAART-treated patients with lipodystrophy: Subcutaneous (n=10)</th>
<th>Visceral (n=8)</th>
<th>p (C vs HIV+)</th>
<th>SAT vs VAT</th>
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<td>Age (y) (+ SD)</td>
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<td>57.5 + 5.9</td>
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</tbody>
</table>

Unless indicated, values are expressed as median (inter-quartile range). BMI, body mass index; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; HOMA-IR, homeostasis model assessment for insulin resistance; p is total p-value between groups; C means controls; HIV+ means HIV-1-infected patients; SAT is subcutaneous adipose tissue; VAT is visceral adipose tissue.
A) mtDNA content

B) Cyt b mRNA

COII mRNA

Cyt c mRNA

COIV mRNA

C) ATPsyn α (Complex V) FeS 30 kd (Complex II)

SAT

VAT

C

LD

C

LD

55 KDa

35 KDa

30 KDa

17 KDa

Cyt b mRNA

COII mRNA

Cyt c mRNA

COIV mRNA

COII (Complex IV)

COIV (Complex IV)