Adipogenic/Lipid, Inflammatory, and Mitochondrial Parameters in Subcutaneous Adipose Tissue of Untreated HIV-1–Infected Long-Term Nonprogressors: Significant Alterations Despite Low Viral Burden

Francesc Vidal, MD, PhD,* Pere Domingo, MD, PhD,† Francesc Villarroya, PhD,‡ Marta Giralt, PhD,‡ Miguel López-Dupla, MD, PhD,* Mar Gutiérrez, MD,† Jose M. Gallego-Escuredo, PhD,‡ Joaquim Peraire, MD, PhD,* Consuelo Viladés, MD, PhD,* Sergi Veloso, MD,* Gracia Mateo, MD,† Jordi P. Guallar, MD,‡ and Cristóbal Richart, MD, PhD*

**Background:** HIV-1 can induce disturbances in adipose tissue in infected subjects through the effects of some of its proteins or inflammation. It is not known whether this also takes place in HIV–1–infected long-term nonprogressors (LTNPs). Our objectives were to determine whether adipocyte differentiation/lipid, inflammatory, and mitochondrial parameters are perturbed in abdominal wall subcutaneous adipose tissue of untreated HIV–1–infected patients LTNPs.

**Methods:** Cross-sectional study involving 10 LTNPs, 10 typical progressors (TPs), and 10 uninfected controls (UCs). The parameters assessed were peroxisome proliferator–activated receptor-gamma (PPARγ), lipoprotein lipase, and fatty acid–binding protein 4 mRNA (adipogenic/lipid); tumor necrosis factor-alpha, interleukin 18 (IL–18), β2-MCG, monocyte chemoattractant protein 1, CD1A, and C3 mRNA (inflammation); and cytochrome c oxidase subunit II (COII), COIV, CYCA, nuclear respiratory factor 1, PPARγ coactivator 1α mRNA, and mtDNA content (mitochondrial).

**Results:** Regarding adipogenic/lipid parameters, LTNPs had PPARγ lipoprotein lipase, and fatty acid–binding protein 4 mRNA significantly decreased compared with UCs (P ≤ 0.01 for all comparisons). PPARγ mRNA was significantly greater in LTNP than in TP (P = 0.006). With respect to inflammatory parameters, tumor necrosis factor-alpha, IL-18, and β2-MCG mRNA were significantly higher in LTNPs compared with UCs (P < 0.005 for all comparisons), whereas IL–18 mRNA was greater in TPs compared with LTNPs (P = 0.01). As mitochondrial parameters are concerned, mtDNA was significantly reduced in LTNPs compared with TPs (P = 0.04) and UCs (P = 0.03). COII and COIV were also significantly reduced in LTNPs compared with UCs and TPs.

**Conclusions:** Adipose tissue from untreated LTNPs may have limited but significant derangements in some adipogenic/lipid and may have inflammatory processes at a lower degree than that observed in untreated TPs. LTNPs may have mitochondrial-related alterations in adipose tissue which are greater than that observed in TPs.

**Key Words:** HIV, long-term nonprogressors, adipose tissue, inflammation, mitochondria

(J Acquir Immune Defic Syndr 2012;61:131–137)

**INTRODUCTION**

Current knowledge suggests that the appearance of the lipodystrophy syndrome in HIV–1–infected patients treated with antiretroviral drugs cannot be explained solely in terms of the effects of the drugs alone and that events related to the HIV–1 infection itself may also be involved.¹ This has been highlighted by the observation that some HIV–1–infected patients who are naïve to antiretroviral drugs may develop adipose tissue disturbances reminiscent of lipodystrophy,²,³ a finding that led investigators to hypothesize that HIV–1 itself could damage adipose tissue. Further experimental evidence supported this. Adipose tissue mtDNA depletion,⁴ impairment of the expression of some mitochondrial genes and of the key master adipogenesis regulator peroxisome proliferator–activated receptor-gamma (PPARγ), and increased expression of some inflammatory mediators such as tumor necrosis factor-alpha (TNF–α) and β2-microglobulin have been shown to be present in untreated HIV–1–infected patients, even without clinical fat redistribution.⁵,⁶ Data elsewhere also suggest that HIV–1 proteins such
as Vpr and Tat are responsible for promoting apoptosis and inhibiting adipogenesis.\textsuperscript{7,12} It has consistently been demonstrated that HIV-1, through its own products and/or secondary inflammatory phenomena, can damage adipose tissue, and that this damage may have clinical consequences. In this respect, we recently performed a study that involved untreated HIV-1–infected patients, and treated patients with lipodystrophy. Subcutaneous adipose tissue (SAT) studies indicated that disturbances in adipose tissue gene expression were present in untreated infected patients, and that these disturbances worsened with the use of antiretroviral drugs, ultimately leading to lipodystrophy.\textsuperscript{3} The subset of untreated HIV-1–infected patients was made up of typical progressors (TPs) (that is to say, individuals with high plasma viral loads and low CD4\textsuperscript{+} T-cell counts). In the present article, we discuss the results of a study performed in HIV-1–infected long-term nonprogressors (LTNPs). LTNPs are a subset of patients characterized by a long-standing HIV-1 infection together with a low viral mass, in the absence of any antiretroviral drug use.\textsuperscript{13} They are regarded as being a model of natural self-control of HIV-1 infection, in which both host and viral factors are involved.\textsuperscript{14}

It can be hypothesized that, because of their lower viral burden, untreated HIV-1–infected LTNPs may have a less disturbed adipogenesis, inflammation, and mitochondrial toxicity parameters in SAT than untreated HIV-1–infected TPs. However, longer exposure to infection, even at a low-grade level, may enhance deleterious processes in adipose tissue in LTNPs. To determine whether this is so, we compared the patterns of gene expression alterations in SAT from HIV-1–infected TPs and LTNPs.

METHODS

Individuals

We performed a cross-sectional case-control study which included 3 patient categories: uninfected controls (UCs), untreated HIV-1–infected LTNPs, and untreated HIV-1–infected TPs. Criteria for LTNPs were asymptomatic HIV-1 infection with a known duration of more than 15 years, a stable plasma HIV-1 viral load repeatedly less than 5000 copies/mL, CD4\textsuperscript{+} count persistently more than 500 cells/\muL, in the absence of any antiretroviral drug use.\textsuperscript{13} Untreated TPs were patients whose HIV-1 infection had progressed (ie, if they had an HIV-1 viral load more than 35,000 copies/mL, and a progressively declining CD4\textsuperscript{+} T-cell count that had gone less than 350 cells/\muL at least twice during the first 8 years of infection) and who had not previously received or were not currently receiving antiretroviral drugs, were free of symptoms, and had not suffered opportunistic infections before recruitment.

Uninfected controls were individuals who had elective abdominal surgery for nonmalignant conditions (usually elective cholecystectomy) and were otherwise healthy. We carefully checked that neither HIV-1–infected patients nor UCs had any conditions known to damage mitochondria, such as smoking, alcohol abuse, or liver disease, and also that they were not taking drugs known to cause mitochondrial derangement.\textsuperscript{15} Informed consent was obtained from each participant. The project was approved by the local ethical research committees.

Methods

Plasma HIV-1 RNA concentrations were measured with the Cobas Amplicor HIV-1 Monitor Test v 1.5 using the COBAS AMPLICOR system (Roche Diagnostics, Basel, Switzerland). Blood CD4\textsuperscript{+} T-cell count was assessed in a flow cytometer FACScan (Becton Dickinson Immunocytometry System, San Jose, CA), and the data acquired were analyzed using the Multiset program.

Subcutaneous fat tissue biopsy was performed in all of the subjects. An 8-mm\textsuperscript{2} sample of abdominal subcutaneous fat was obtained by biopsy. Tissue samples were frozen in liquid nitrogen and kept at −80°C until processing. After homogenization in RLT buffer (Qiagen, Hilden, Germany), an aliquot was used to isolate DNA, with a standard phenol/chloroform extraction methodology. RNA extraction was performed using a column-affinity–based methodology (RNasy; Qiagen), which included on-column DNA digestion (RNase-Free DNase set, Qiagen). One milligram of RNA was transcribed into cDNA using MultiScribe reverse transcriptase and random-hexamer primers (TaqMan Reverse Transcription Reagents; Applied Biosystems, Foster City, CA). For quantitative mRNA expression analysis, 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 \muL using TaqMan Universal PCR Master Mix, No AmpErase UNG reagent, and the specific gene expression primer pair probes (Applied Biosystems).

The parameters assessed and their biological significance are detailed in Table 1. The assay-on-Demand probes (TaqMan Gene Expression Assays; Applied Biosystems) used were 18S rRNA, Hs009999901; cytochrome c oxidase subunit IV (COIV), COX4I1, Hs00266371; nuclear respiratory factor 1, Nrf1, Hs00173304; lipoprotein lipase (LPL), Hs00173425; fatty acid–binding protein 4 (FABP4), Hs00609791, TNF-\alpha, Hs00174128; monocyte chemoattractant protein 1 (MCP-1), Hs00234140; interleukin 18 (IL-18), Hs009999040; complement component 3 (C3), Hs01638111; CD1A, Hs00381753; \beta2-microglobulin, Hs009999907. The primers and probes for detecting COII and mtDNA abundance assessment were designed using the Assay-by-Design system (Custom TaqMan Gene Expression Assays; Applied Biosystems), and the sequence were AAACACATTTCACCCTACGCTAC (forward) and GGAGCATGAGGGCATAGAACTGT (reverse). The FAM-labeled probe was AAATCTGTGGAGCAAACC. mtDNA was quantified using these last probe and referred to nuclear DNA as determined by the amplification of the intronless gene C/EBPalpha (Hs00269972), as previously reported.\textsuperscript{16} 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 the duplicate was used to calculate the mRNA expression of the genes of interest which were normalized to that of the reference control (18S rRNA) using the comparative (2-DeltaCT) method and following the instructions of the manufacturer. Parallel calculations using the cyclophilin-A (peptidyl propylisio reverse A [PPIA]) reference gene (Hs99999904), another housekeeping gene, were performed and results were essentially the same.
Table 1. Explanation and Significance of the SAT mRNA Parameters Assessed

<table>
<thead>
<tr>
<th>Parameter Assessed</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adipogenic/lipid</strong></td>
<td></td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator–activated receptor-gamma. Key master regulator of adipocyte differentiation</td>
</tr>
<tr>
<td>FABP4</td>
<td>Fatty acid–binding protein 4. Transports fat into the adipocyte. It is a PPARγ target</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase. Captures fatty acids by adipose tissue. It is a PPARγ target</td>
</tr>
<tr>
<td><strong>Inflammatory</strong></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-alpha. Proinflammatory cytokine</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein 1. Proinflammatory cytokine</td>
</tr>
<tr>
<td>IL-18</td>
<td>Interleukin 18. Proinflammatory cytokine</td>
</tr>
<tr>
<td>CD1A</td>
<td>Dendritic cell marker</td>
</tr>
<tr>
<td>β2-microglobulin</td>
<td>Target for TNFα and other proinflammatory cytokines</td>
</tr>
<tr>
<td>C3</td>
<td>C3 component of the complement system</td>
</tr>
<tr>
<td><strong>Mitochondrial</strong></td>
<td></td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA content</td>
</tr>
<tr>
<td>COII</td>
<td>Subunit II of the cytochrome oxidase. Member of the mitochondrial respiratory chain encoded by the mitochondrial DNA</td>
</tr>
<tr>
<td>COIV</td>
<td>Subunit IV of the cytochrome oxidase. Member of the mitochondrial respiratory chain encoded by the nuclear DNA</td>
</tr>
<tr>
<td>NRF1</td>
<td>Nuclear respiratory factor 1. Mitochondrial biogenesis regulator</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Peroxisome proliferator–activated receptor (PPAR) gamma coactivator 1α. Transcriptional coactivator involved in mitochondrial biogenesis</td>
</tr>
</tbody>
</table>

Statistical Analysis

Data of continuous variables of all categories were expressed as median and 25th and 75th percentiles. The Kruskal–Wallis test was performed to compare the continuous variables of the 3 categories: UC, LTNP, and TP, and the Mann–Whitney U test was performed to compare the results between 2 groups. The χ² test, with Yates if necessary, was performed to compare discrete variables. In all cases, a P value of less than 0.05 was considered to be statistically significant.

RESULTS

Characteristics of the Participants

Table 2 shows the main characteristics of the UCs and the 2 categories of HIV–1–infected patients studied. Groups were comparable for age and gender. As expected, the duration of HIV–1 infection, the plasma viral load, and CD4⁺ T-cell count were significantly different between HIV–1–infected LTNPs and TPs (Table 2). As shown, our untreated LTNPs were individuals with extreme long-standing infection (more than 15 years) with preserved immune parameters and low viral burden.

Adipogenic/Lipid Parameters

Data are shown in Figure 1. Overall, PPARγ, FABP4, and LPL mRNA expression were significantly decreased in both HIV–1–infected subsets with respect to UCs. Comparison of HIV–1–infected LTNPs and HIV–1–infected TPs indicated that the latter had a significantly decreased mRNA expression of PPARγ (P = 0.006). LPL and FABP4 expression were nonsignificantly different between these 2 subsets.

Inflammatory Parameters

In abdominal wall SAT, TNF–α, IL–1β, IL–18, MCP–1, COII, COIV, CD1A, and C3 mRNA were assessed (Fig. 2). The behavior of the various parameters assessed was somewhat different. TNF–α, IL–1β, and IL–18 showed the following trend: both HIV–1–infected subsets had significantly greater mRNA expression than UCs. In HIV–1–infected subjects, IL–18 was significantly greater in TPs than in LTNPs, and TNF–α and β2-MCG were nonsignificantly different in these 2 subsets. C3 was significantly increased in UCs with respect to both HIV–1–infected subsets. Otherwise, CD1A and MCP–1 showed a similar trend to C3, but the differences were nonsignificant (Fig. 2).

Mitochondrial Parameters

We assessed mtDNA content and COII and COIV mRNA levels. The last 2 are mitochondrial respiratory chain proteins encoded by mitochondrial and nuclear DNA, respectively. COII and COIV were significantly lower in LTNP than in UCs and TPs. TPs had lower COII and COIV mRNA expression than UCs, but the difference was significant only for COIV. This may reflect the effect of HIV–1 on diffuse cell damage. The levels of mtDNA in adipose tissue were significantly lower in LTNPs respect to UCs, but not in TPs (Fig. 3). In fact, mtDNA levels in LTNPs were significantly lower than in TPs. There were no significant differences between the 3 subsets of individuals for the transcript levels of the mitochondrial biogenesis regulator nuclear respiratory factor 1. The mRNA expression for the PPARγ coactivator 1α, coactivator of mitochondrial biogenesis, was significantly increased only in TP.

DISCUSSION

Previous studies have demonstrated that HIV–1 infection itself produces mitochondrial disturbances in the peripheral blood mononuclear cells of untreated infected patients. It has also been reported that disturbance is lower in LTNP than in TP. In the present study, we demonstrate for the first time that this also occurs in adipose tissue, since untreated HIV–1–infected LTNP may have limited but significant derangement in some adipogenic/lipid, inflammatory, and mitochondrial parameters in SAT. This derangement is lower than that observed in untreated TPs, with the exception of mitochondrial damage. Hence, our data suggest that HIV–1 itself may damage adipose tissue and that the derangement correlate, at least partially, with both the viral burden and the duration of infection. Whether the derangement of
adipogenic, inflammatory, and mitochondrial parameters in adipose tissue starts shortly after infection or develops over time remains unknown.

Our observations should be borne in mind when studying the pathogenesis of lipodystrophy, the exact mechanisms of which still remain obscure. At one time, lipodystrophy was thought to be an adverse effect of protease inhibitors, because of the close relationship between the widespread use of this drug family and the recognition of the syndrome. To date, the reason why some protease inhibitors produce lipodystrophy (and particularly the lipohypertrophic component of the syndrome) has not been satisfactorily explained, but perturbations in the process of adipogenesis have been proposed.

It has also been shown that some nucleoside reverse transcriptase inhibitors are also associated with lipodystrophy (and especially with lipoatrophy). The association is particularly strong with thymidine analogues—zidovudine and, especially, stavudine—which is thought to be because of the mitochondrial toxic potential of these drugs, which favors adipocyte apoptosis. Thus, the role of antiretroviral drugs in lipodystrophy has consistently been demonstrated, although there is no consensus on which molecular mechanisms are involved. Otherwise, currently available data suggest that HIV-1 itself could also be involved in adipose tissue derangements. In this respect, basic science studies have shown that some proteins produced by the virus (Vpr, Tat, Nef, among others) may have deleterious effects on the adipose tissue. Moreover, HIV-1 transgene expression in mice causes changes in adipose tissue that are reminiscent of those in patients with lipodystrophy, particularly early pretreatment changes. Clinical association studies confirmed that HIV-1–infected subjects, in the absence of any antiretroviral treatment, show marked derangements in adipose tissue, which include an increased local inflammatory milieu and perturbations in adipogenesis and mitochondrial parameters. Before the present study, it was not known whether the pattern and intensity of the derangement produced by HIV-1 in adipose tissue varies in different clinical patterns of the infection, but we have provided here evidence that suggests that even a low-invasive pattern of infection (represented by the untreated LTNPs) damages adipose tissue, although to a lesser extent than untreated TPs. A consistently low viral burden over a long period of time, as is the case of LTNPs, produces significant adipose tissue derangement.

Table 2. Demographic and HIV-1–Related Data of the Individuals Assessed. Data are Expressed as Median (25th–75th)

<table>
<thead>
<tr>
<th>Variable</th>
<th>UCs (n = 10)</th>
<th>Untreated HIV-1–Infected Long-Term Nonprogressors (n = 10)</th>
<th>Untreated HIV-1–Infected Typical Progressors (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (±SD)</td>
<td>37 (32.8–40.5)</td>
<td>42 (38–43)</td>
<td>36 (29–41.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Sex (male, %)</td>
<td>80%</td>
<td>50%</td>
<td>80%</td>
<td>0.24</td>
</tr>
<tr>
<td>Time since HIV-1 infection diagnosis (yrs)</td>
<td>—</td>
<td>16 (15.6–17.6)</td>
<td>4 (3–8.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HIV-1 RNA (log_{10} copies per milliliter)</td>
<td>—</td>
<td>1.7 (1.3–3.3)</td>
<td>4.8 (4.6–5.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+ T-cell count (cells per cubic millimeter)</td>
<td>—</td>
<td>658 (581–782)</td>
<td>296 (180–328)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

FIGURE 1. Adipocyte differentiation and lipid metabolism marker gene expression in SAT from UCs, untreated HIV-1–infected LTNPs, and untreated HIV-1–infected typical progressors (TPs). The figure shows the box-and-whisker plot representing specific mRNA concentrations. The line within the box marks the median, the upper boundary of the box indicates the 75th percentile, and the lower boundary the 25th percentile. Error bars above and below the box indicate the 100th and 0th percentiles. For comparison, the P values are indicated above the boxes when statistical significance is P < 0.05.
A detailed analysis of our observations suggests that HIV-1 itself markedly impairs the expression of proteins involved in adipocyte differentiation, such as PPARγ, LPL, and FABP4. The duration of the infection and the amount of viral mass do not seem to affect LPL and FABP4, whereas a long-standing infection reduces PPARγ expression. This suggests that this key master regulator of the adipocyte differentiation process is more sensitive to the duration of the infection than to the viral mass.

As far as inflammatory parameters are concerned, we and others have observed that the inflammatory milieu is increased in the subcutaneous fat of treated infected patients with lipodystrophy. Giralt et al reported that HIV-1 infection itself produces some degree of inflammation in adipose tissue. We have shown here that this already exists in LTNPs, although to a lesser extent, particularly with respect to IL-18. Our findings suggest that even a low-invasive HIV-1 infection, exemplified by LTNPs, produces some degree of inflammatory derangement in the host. Because inflammation has been correlated with lipodystrophy and both premature atherosclerosis and aging, our data seem to suggest that clinical studies should consider the possibility of prescribing antiretroviral treatment earlier than that is currently recommended in guidelines.

As far as the disturbances in the adipose tissue mitochondrial parameters assessed in our study are concerned, the data confirm that untreated HIV-1–infected patients show mild mitochondrial damage in adipose tissue. We have also observed that long-standing low-invasive HIV-1 infection (the LTNP model), alters mitochondrial-related parameters more markedly than a shorter infection with higher viral mass (the TP model). This suggests that mitochondrial damage is related more to the duration of the infection than to the amount of the infection (viral mass), as occurs with other causes of mitochondrial derangement. In fact, distinct pathologies caused by mitochondrial alterations (ie, mtDNA genetic diseases) are usually progressive degenerative diseases, with a marked worsening of symptoms over time. We should point out that in our groups of patients, despite a trend to lower mtDNA levels in adipose tissue from TP, only LTNP showed a significant depletion of mtDNA with respect to UC. There have been reports of unaltered mtDNA levels or mtDNA depletion in HIV-1–infected TP. Maybe these varied findings could be explained by slight differences in the duration of HIV-1 infection in the untreated patients assessed.

We acknowledge that our study has some limitations. First, the cross-sectional nature of our design provides associations, not causality. Second, there are some inconsistencies in our results because as we have discussed above, some parameters were damaged, others were not, and others were by means no clear or difficult to interpret because of considerable variation in some subsets. Of note, the data for C3, CD1A, and MCP-1 in UC varied considerably, and this may render comparisons somewhat difficult to interpret. Third, the low number of patients assessed suggests prudence when interpreting our data. Some nonsignificant comparisons (because of underpower) might become significant if replicated in further studies.

FIGURE 2. Inflammation-related marker gene expression in SAT from UCs, untreated HIV-1–infected LTNPs, and untreated HIV-1–infected TPs. The figure shows the box-and-whisker plot representing specific mRNA concentration. The line within the box marks the median, the upper boundary of the box indicates the 75th percentile, and the lower boundary the 25th percentile. Error bars above and below the box indicate the 100th and 0th percentiles. For comparison, the P values are indicated above the boxes when statistical significance is P < 0.05.
larger studies. It should be highlighted, however, that it is not easy to perform a study such as this because it is difficult to convince individuals who are completely asymptomatic and not taking antiretroviral drugs to permit a SAT biopsy.

In summary, the present study suggests that HIV-1 by itself may damage the expression of diverse adipogenic/lipid, inflammatory, and mitochondrial parameters in adipose tissue, and that this is even true in patients with an extremely low-invasive pattern of infection, such as LTNP. These patients, although they have low-grade viremia, may have significant mRNA gene adipose-tissue disturbances.

ACKNOWLEDGMENTS

We would like to thank our patients who kindly provided samples of SAT. John Bates improved the English text. The comments and criticisms performed by the anonymous reviewers helped us to improve the manuscript.

REFERENCES


