Structured Intermittent Interruption of Chronic HIV Infection Treatment with Highly Active Antiretroviral Therapy: Effects on Leptin and TNF-α

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ABSTRACT

The changes in nutritional parameters and adipocytokines after structured intermittent interruption of highly active antiretroviral treatment of patients with chronic HIV infection are analyzed. Twenty-seven patients with chronic HIV infection (median CD4+ T cell count/μl: nadir, 394; at the beginning of structured interruptions, 1041; HIV viral load: nadir, 41,521 copies/ml; at the beginning of structured interruptions <50 copies/ml; median time of previous treatment: 60 months) were evaluated during three cycles of intermittent interruptions of therapy (8 weeks on/4 weeks off). CD4+ T cell count, HIV viral load, anthropometric measures, and serum concentrations of triglycerides, cholesterol, leptin, and tumor necrosis factor and its soluble receptors I and II were determined. After the three cycles of intermittent interruptions of therapy, no significant differences in CD4+ T cell count/μl, viral load, or serum concentrations of cholesterol or triglycerides with reference to baseline values were found. A near-significant higher fatty mass (skinfold thicknesses, at the end, 121 mm, at the beginning, 100 mm, p = 0.100), combined with a significant increase of concentration of leptin (1.5 vs. 4.7 ng/ml, p = 0.044), as well as a decrease in serum concentrations of soluble receptors of tumor necrosis factor (TNFRI, 104 vs. 73 pg/ml, p = 0.022; TNFRII 253 vs. 195 pg/ml, p = 0.098) were detected. Structured intermittent interruption of highly active antiretroviral treatment of patients with chronic HIV infection induces a valuable positive modification in markers of lipid turnover and adipose tissue mass.

INTRODUCTION

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) significantly reduces mortality due to human immunodeficiency virus (HIV) infection.1 However, the main goal of HAART, i.e., the eradication of the virus, has not been achieved.2 Thus, HAART needs to be continued for the remainder of a patient’s life. This prospect creates several problems, such as the high cost of therapy, decreased adherence, and drug-related side effects. One of the more frequent HAART-induced toxicities is a syndrome of peripheral fat wasting, central adiposity, hyperlipidemia, and insulin resistance, referred to as lipodystrophy syndrome.3 An association between lipodystrophy and treatment with nucleoside reverse transcriptase inhibitors, possibly accelerated in the presence of a protease inhibitor, has been demonstrated.3

It is thought that adipose tissue performs at least two major functions, namely the storage and release of energy-rich fatty acids and the secretion of proteins involved in the endocrine or autocrine regulation of energy metabolism and insulin sensitivity.4 Two of the most intensely investigated proteins secreted by adipose tissue (adipocytokines) are leptin and tumor necrosis factor (TNF)-α. Correlations are found between the amount of body fat and the circulating levels of leptin, suggesting that leptin might act as a part of a feedback mechanism signaling the brain about the amount of fat stored in the body and regulating appetite and energy balance.5,6 For its part, TNF-α is a proinflammatory cytokine, secreted by diverse cells, with effects on lipid and glucose metabolism: TNF-α inhibits the intake of free fatty acids by adipocytes via the inhibition of lipoprotein lipase, which leads to fat wasting, and increases lipogenesis via stimulation of the hepatic triglyceride synthetase, which leads to hyperlipidemia7; accumulation of TNF-α could also account for insulin resistance, both indirectly via increases of free fatty acids and directly via the inhibition of signal transduction through the insulin receptor.7

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Site differences in lipolysis, as well as in the synthesis of and response to adipokines, have been demonstrated. Thus, the leptin production rate is much higher in subcutaneous than in omental adipose tissue. Regional variation might play a role in controlling the size of various fat depots. In contrast, adipose TNF-α secretion does not seem to be subject to depot-specific differences, although higher expression levels of TNF-α receptors have been found in subcutaneous versus omental adipose tissue.

Given the fat accumulation or fat wasting that occurs in HIV-infected patients with lipodystrophy, it has been hypothesized that adipocyte function may play an important role in the development of the associated metabolic abnormalities. The influence of proinflammatory cytokines and adipokines has been analyzed. Thus, although controversial data exist, an increase of TNF-α and a decrease of leptin have been demonstrated in this condition. Fat loss by HIV-infected patients with lipodystrophy has been associated with low levels of leptin, increased visceral fat, insulin resistance, and hypertriglyceridemia. Moreover, subcutaneous fat atrophy is associated with focal lipogranuloma formation and adipocyte apoptosis, which is compatible with excessive local production of TNF-α. In addition, TNF-α may also be involved in insulin resistance and hyperlipidemia.

Thus, although HAART provides extraordinary clinical benefit, there is an unmet need for alternative therapies to continuous HAART so as to enhance adherence and to reduce costs and toxicities. One strategy in particular that has been studied is structured treatment interruptions (STI), comprising alternating on and off cycles of HAART. The theory initially supporting STI in individuals who have been successfully treated with HAART was to allow short bursts of plasma viremia to HIV-specific immune responses. However, in patients with chronic HIV infection, it has not been proved that STI provides a significant benefit with regard to controlling HIV viral load, to preserving levels of CD4+ T cell counts, or to increasing the HIV-immune-specific responses. A lower exposure to antiretrovirals, as when STI is implemented, could limit the secondary effects of treatment. Limited data have demonstrated that short-cycle (7 days on/7 days off) STI significantly reduces serum total and LDL cholesterol and serum triglyceride levels. Levels of these lipids did not significantly change during a long-cycle (8 weeks on/4 weeks off) STI. Modifications in other parameters related to lipodystrophy, such as anthropometric measures, TNF-α activity, or leptin, have not been evaluated in this situation. Accordingly, in an attempt to clarify the nature of in vivo interactions between HAART and the production of adipokines, we have assessed changes in plasma viral load, peripheral blood CD4+ T cell counts, and circulating levels of TNF-α, TNF-receptors, and leptin in a group of HIV-infected patients after applying STI.

### MATERIALS AND METHODS

#### Patients

Twenty-seven patients with chronic HIV infection were analyzed. Inclusion criteria were (1) a minimal CD4+ T cell count, previous to receiving HAART, higher than 350/µl; (2) a CD4+ T cell count higher than 500/µl at the beginning of the study; (3) an HIV RNA level lower than 50 copies/ml during the previous 6 months; and (4) patients had to be receiving HAART, consisting of at least a three-drug regimen, for at least 12 months previously. Exclusion criteria were (1) patients should not have had a history of opportunistic infections and (2) their clinical history should not have been compatible with loss of adherence. None of the patients had a history of endocrine disorders, obesity, or decompensated hepatic disease at the time of inclusion or during the follow-up and none was on lipid-lowering therapy. Characteristics of patients are presented in Table 1. For the analysis of leptin, TNF-α, and its soluble receptor concentrations, a sample of 20 aged-matched and sex-matched healthy individuals (median age 34, interquartile range 30–38 years; male:female ratio 8:2; body mass index 23; interquartile range 22–25 kg/m²), in whom the absence of evidence of disease was demonstrated, was selected as controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>37 (33–42)</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>18/9</td>
</tr>
<tr>
<td>Time from diagnosis of HIV infection until the beginning of study (months)</td>
<td>84 (60–120)</td>
</tr>
<tr>
<td>Minimum CD4+ T cell count/µl</td>
<td>394 (358–642)</td>
</tr>
<tr>
<td>CD4+ T cell count/µl at the beginning of the study</td>
<td>1041 (678–1413)</td>
</tr>
<tr>
<td>Maximum HIV viral load (mean ± SD), copies/ml × 1000</td>
<td>42 (15–373)</td>
</tr>
<tr>
<td>Number of HAART regimens previous to actual regimen</td>
<td>1 (0–2)</td>
</tr>
<tr>
<td>Time in HAART regimen</td>
<td>60 (48–72)</td>
</tr>
<tr>
<td>Actual HAART regimen</td>
<td></td>
</tr>
<tr>
<td>Zidovudine + lamivudine + indinavir</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>Zidovudine + didanosine + nelfinavir</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>Zidovudine + didanosine + ritonavir + saquinavir</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>Zidovudine + lamivudine + lopinavir</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Stavudine + lamivudine + indinavir</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>Stavudine + lamivudine + nelfinavir</td>
<td>3 (11.1)</td>
</tr>
</tbody>
</table>

aData are presented as median (interquartile range) or as number (percentage).
Study schedule

The schedule of STI included 8 weeks with HAART and 4 weeks without it. At the end of each phase (on and off), medical visits were programmed, including clinical history and physical examination (specifically, an active search for HIV-related opportunistic diseases and acute retroviral syndrome, was performed); anthropometric measures, hemogram, determination of lipid fractions, leptin, TNF-α, and its receptors were observed during STI.

Because of concern about the development of potential resistance and/or loss of efficacy of HAART, safety criteria were instituted. First, patients were not eligible if they had ever changed treatment because of virological failure. Second, an analysis of the treatment response was performed after each cycle. HAART would be discontinued for another period of time only in patients whose plasma viral load had decreased to <50 copies/ml after therapy reinitiation. The trial would be stopped if, among the 27 patients, two or more failed to suppress viremia to <50 copies/ml upon reinitiating the therapy.

The study protocol was approved by the Institutional Ethics Committee and all patients gave their informed consent.

Nutritional assessment

Lipodystrophy was defined in patients if they had at least one of the following: peripheral fat wasting (face, arms, buttocks, or legs), weight gain confined to the abdomen or breast enlargement in women, or buffalo hump, according to accepted criteria from the literature. These findings were confirmed by the investigators.

The following anthropometric variables were determined: body mass index [weight in kilograms / height in meters²], waist circumference (measured in centimeters midway between the lower rib margin and the iliac crest), hip circumference (measured in centimeters over the greater trochanters), skinfold thicknesses (sum of mean values of triplicate measures determined at five sites: triceps, biceps, subscapular, abdominal, and thigh), and arm muscular area [(brachial perimeter – 3.14 x triceps skin fold)²/4 x 3.14]. Anthropometrical parameters were measured as described elsewhere. All tests were performed by a single investigator (GI). The intraobserver variability in reading anthropometric parameters was less than 6.5%. The reproducibility of these measures was initially proved in our work by repeating the same test sequence on two consecutive days in the 27 patients.

Laboratory determinations

Measurements of fasting serum glucose, uric acid, cholesterol, and triglyceride concentrations were performed according to standard methods with the use of automated equipment (Hitachi, Boehringer Mannheim, Indianapolis, IN). CD4⁺ T cell counts were determined by standard flow cytometry. Serum HIV load was determined by reverse transcription polymerase chain reaction (AmpliFlor HIV, Roche Diagnostics, Basel, Switzerland). Sensitivity of the assay is 50 RNA copies per ml of serum.

Blood samples, collected in sterile Vacutainer tubes (Becton-Dickinson Vacutainer System, Meylan Cedex, Frac), were centrifuged (3500 rpm for 15 min at 4°C) and serum stored at −80°C in pyrogen-free polyethylene tubes (Biofreeze, Costar, Cambridge, MA) until leptin, TNF-α, and soluble receptors of TNF were assayed. Serum levels of leptin, TNF-α, sTNFRI (TNFp55), and TNFRII (TNFp75) were assayed with ELISA kits (R & D, Minneapolis, MN), according to manufacturer’s instructions, with the following detection limits (lowest positive standard): leptin, 0.78 ng/ml; TNF-α, 15 pg/ml; sTNFRI, 7.8 pg/ml; sTNFRII, 7.8 pg/ml.

Statistical analysis

Data are presented as median and interquartile range or, when indicated, as absolute number and percentage. The data from two independent groups were compared with the Mann–Whitney U test. Significance of parameters within each group was tested by a repeated measures ANOVA. For qualitative variables, X² with Yates’ correction or Fisher’s exact test was used. A p value lower than 0.05 was considered significant. The statistical analysis was performed using the SPSS 11.0 (SPSS Inc., Chicago, IL) and STATA 7.0 (Stata Corp, College Station, TX) programs.

RESULTS

Evolution of clinical, immunological and virological parameters of the patients

Twenty-seven patients were enrolled in the study. They were assigned to receive long-cycle STI of 4 weeks without HAART followed by 8 weeks with HAART. Three of these cycles were analyzed. No major (CDC class C) or minor (CDC class B) HIV-related opportunistic diseases were observed during STI. Likewise, an acute retroviral syndrome was not detected in any of the cases. Table 2 shows the evolution of CD4⁺ T cell count and plasma HIV viral load in the patients. Differences between CD4⁺ T cell counts at the beginning and at the end of the study were not significant. HIV viral load decreased to <50 copies/ml after therapy reinitiation in each of the three STI cycles.

TNF-α, TNF-α receptors, and leptin

HIV-infected patients showed significantly higher serum concentrations of soluble TNF-R1 and sTNF-R2, but not of TNF-α, compared with healthy controls [median (interquartile range) values, TNF-α: 0.7 (0–1.1) vs. 0.6 (0–0.9) pg/ml, p = 1.00; TNF-R1: 104.1 (66.0–143.2) vs. 12.0 (6.0–15.9) pg/ml, p < 0.001; TNF-R2: 253.2 (154.5–412.3) vs. 16.3 (13.4–21.0) pg/ml, p < 0.001]. Serum leptin concentration was significantly lower in HIV-infected patients than in healthy controls [1.5 (1.1–2.2) vs. 3.4 (2.9–4.3) ng/ml, p = 0.034].

Figure 1 shows the concentrations of TNF-α and its receptors, as well as the concentrations of leptin, during the follow-up of these patients. A significant decrease of serum concentrations of soluble TNF-R1 (p = 0.022) and a near-significant decrease of those of TNF-R2 (p = 0.098) were detected between values at baseline and at the end of the study. Concentrations of leptin were significantly higher at the end of follow-up compared with the baseline values (p = 0.044).

At the end of follow-up, although the serum concentrations of soluble TNF receptors continued to be significantly higher than those of healthy controls (p < 0.001 in each case), the
<table>
<thead>
<tr>
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<th>First cycle</th>
<th>Second cycle</th>
<th>Third cycle</th>
<th>End of study</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HAART on</td>
<td>HAART off</td>
<td>HAART on</td>
<td>HAART off</td>
</tr>
<tr>
<td>CD4⁺ T cell count/μl</td>
<td>1041 (678–1413)</td>
<td>731 (522–988)</td>
<td>968 (620–1403)</td>
<td>788 (515–1130)</td>
</tr>
<tr>
<td>HIV viral load, copies/ml × 1000</td>
<td>&lt; 50 (3–12)</td>
<td>14 (2–59)</td>
<td>&lt; 50 (2–59)</td>
<td>12 (2–59)</td>
</tr>
<tr>
<td>Serum triglycerides concentration (mg/dl)</td>
<td>158 (132–287)</td>
<td>133 (95–220)</td>
<td>153 (110–250)</td>
<td>124 (105–324)</td>
</tr>
<tr>
<td>Serum glucose concentration (mg/dl)</td>
<td>95 (91–101)</td>
<td>89 (82–106)</td>
<td>95 (89–97)</td>
<td>89 (86–94)</td>
</tr>
<tr>
<td>Serum uric acid concentration (mg/dl)</td>
<td>6.1 (4.2–7.5)</td>
<td>5.9 (4.6–7.4)</td>
<td>5.7 (4.9–6.5)</td>
<td>6.4 (5.4–7.1)</td>
</tr>
</tbody>
</table>

aData are presented as median (interquartile range).
serum leptin levels were similar to those of the healthy subjects ($p = 0.431$).

**Anthropometric measures**

Figure 2 shows the evolution of anthropometric measures during three STI cycles. Although there were statistically significant differences between the phases with HAART and without HAART in each of the cycles, with respect to body mass index, the difference between the value of this parameter at enrollment and at the end of the study was not significant ($p = 0.337$). A progressive increase of the sum of skinfold thickness, from 100 at enrollment to 121 mm at the end of the study, was detected, approaching statistical significance ($p = 0.100$). In accordance with previously accepted criteria, lipodystrophy was diagnosed in 33.3% ($n = 9$) of our patients. Patients did not subjectively note any differences in areas of fat wasting (face, arms, buttocks, or legs) or fat gain (abdomen or breast).

Changes of concentrations of leptin, TNF-$\alpha$, or its receptors after STI were not significantly different in patients with or without lipodystrophy (data not shown). Concentrations of leptin were correlated with the sum of skinfold thickness ($r = 0.3$, $p = 0.04$). Concentrations of TNF-$\alpha$ or of its receptors were not correlated with any of the other parameters evaluated.

**Serum lipid levels**

Table 2 shows the evolution of serum concentrations of total cholesterol and triglycerides, as well as those of glycemia and uricemia. Again, although a decrease of concentrations of cholesterol and triglycerides was detected when HAART was suppressed, the differences between values at baseline and at the end of the study did not reach statistical significance.

**DISCUSSION**

The present study has analyzed the effect of long-cycle STI on two sets of parameters: those related to HIV infection (clinical findings, CD4$^+$ T cell counts and plasma HIV viral load) and those related to the secondary effects of the therapy. Fischer et al. recently demonstrated that 81.5% of patients presented with significant replication 14 days after suspension of HAART, with the consequent increase in the risk of emergence of drug resistance. These and other data from the analysis of short-cycle or long-cycle STI raise doubts about STI in chronic HIV-1 infection. In contrast with these recent articles analyzing this topic, none of our patients included in long-cycle STI failed to suppress viremia to $<50$ copies/ml.
upon reinitiation of therapy, a finding similar to that previously reported by other authors.35,36 This could be due to the differences between the patients of Dybull et al.26 or of Fagard et al.34 and ours with regard to previous HAART therapy and, consequently, with resistance to the treatment. However, our data, as well as those of other authors,20–27 have not demonstrated an increase in CD4+ T cell count or a better control of HIV viral load with successive interruptions of the therapy. In our series of patients, a decrease of CD4+ T cell count (from a median of 1041 to a median of 807 cells/mm³) was detected during the follow-up, although this difference did not reach statistical significance. Moreover, no significant differences in HIV-1 viral load were detected between values at baseline and at the end of our study.

The other aspect considered in our article was the possibility of controlling HAART-induced adipose tissue and lipid alterations. These abnormalities include peripheral fat wasting, central adiposity, and hyperlipidemia.3,11 It has been established that in HIV-infected patients with HAART-induced lipodystrophy, the serum concentrations of leptin, a hormone produced by adipose tissue, are reduced.13–17 Likewise, in HIV-infected patients with or without HAART, serum concentrations of TNF-α and its receptors are increased.13–17,37 Similar differences were observed between our patients and controls.

Long-cycle STI, as applied in this study (8 weeks on/4 weeks off), reduces the exposition to antiretrovirals by one-third of the total time. Although a decrease in serum lipid fractions (total cholesterol and triglycerides) was observed in each of the periods without HAART, at the end of the study period (three cycles), no evidence of analytical differences was observed compared with values at baseline. It is unclear whether these periodic reductions in lipid levels could ultimately result in a clinical benefit to patients over time.

Markers of lipolysis, such as those derived from the TNF-α system, or of adipose tissue mass, such as leptin, were modified during STI. A decrease of serum levels of TNF receptors was detected, although concentrations did continue to be higher than those detected in healthy controls. The multiple activities of TNF-α are mediated through two high-affinity receptors, TNFRI and TNFRII.38 Circulating soluble TNFRs, derived by

FIG. 2. Anthropometric measures (body mass index, waist and hip circumferences, arm muscular area, and sum of skinfold thickness—sum of mean values determined at five sites: triceps, biceps, subscapular, abdominal, and thigh) in patients with chronic infection by human immunodeficiency virus after three cycles of structured intermittent interruption of highly active antiretroviral treatment (8 weeks on/4 weeks off).
proteolytic cleavage from the extracellular portions of their respective membrane-associated TNFRs, are thought to reflect in vivo TNF-α activity.38,39 Because they have a longer mean life, their determination provides more accuracy in the analysis of the TNF-α system.31 Values of TNF receptors decrease with each interruption cycle, with values clearly different at the end of the study when compared with those from the beginning. This result could appear paradoxical: an increase of TNF-α and of its receptors could be expected from the increase of HIV load and immune stimulation.40 However, recent reports have demonstrated that HAART is associated with a suppression of apoptosis in TNF-α cell producers, with the progressive accumulation of T cells producing TNF-α, thereby creating a proinflammatory environment that might contribute to the development of lipid abnormalities.31–43 The cycles of HAART interruption probably produce the disappearance of apoptosis suppression in these T cells, thus decreasing TNF-α synthesis.

An inverse correlation between serum levels of leptin and TNF receptors has been demonstrated in HIV-infected patients.44 Accordingly, in our study, the decrease of soluble TNF receptor levels was associated with an increase of leptin concentration. Leptin is a marker of adipose tissue mass. The increased leptin levels seen in our patients may signify a state of higher production by increased adipose tissue mass,13,15 as a consequence of a reduced exposure to antiretrovirals. It is notable that at the end of follow-up, serum leptin levels in HIV-infected patients were similar to those detected in healthy controls.

It has been hypothesized that leptin has a critical role in preventing insulin resistance and hypertriglyceridemia of lipodystrophy.45 Interestingly, leptin replacement therapy improves glycemic control and decreases triglyceride levels in patients with other forms of lipodystrophy different from that associated with HIV infection.45 In contrast, TNF-α inhibits lipoprotein lipase and stimulates hepatic triglyceride synthetase, which lead to fat wasting and hyperlipidemia, respectively.7 Likewise, TNF-α favors an insulin-resistant state, via the increase of free fatty acids and inhibition of signal transduction through the insulin receptor.7 Thus, the increase of leptin and decrease of soluble TNF receptor levels together provide a favorable scenario for fat gain and, possibly, an amelioration of lipoatrophy in HIV-infected patients treated with antiretrovirals.

Site differences in lipolysis, as well as in the synthesis of and response to adipocytokines, have been demonstrated. Thus, the leptin production rate is much higher in subcutaneous than in omental adipose tissue.8–10 This regional variation might play a role in controlling the size of different fat depots, with a near significant increase in subcutaneous fat depots (sum of skinfold thickness), but not in abdominal perimeter, in treated patients, as was observed in our study. It is possible that modifications in biochemical parameters are not translated into anthropometric changes in only 9 months of STI (a total of 3 months without HAART) and more prolonged follow-up will be needed.

Results in respect to lipid markers should be balanced with the absence of immunological and virological efficacy of long-cycle STI and, if a benefit in terms of immunological or virological parameters was not evident, this treatment must be questioned. Changes in the current guidelines for beginning the treatment could discount STI in patients with an acceptable level of immunocompetence and low viral load. Other approaches, such as prolonged interruption of treatment until a CD4+ T cell count lower than 350/μl or an HIV viral load >50,000 copies/ml is reached, could be acceptable.47 Larger increases of leptin concentrations after a long-term suppression of antiretrovirals must be expected.

REFERENCES


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