

# Serum Leptin Levels In Hiv-1 Infected Nigerians Receiving Highly Active Antiretroviral Therapy

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## Abstract

**INTRODUCTION:** The possible effects of highly active antiretroviral therapy (HAART) on serum leptin levels were determined by comparing serum leptin levels in HAART naïve and HAART experienced normal weight HIV infected patients. **METHODS:** A cross sectional study was undertaken in Ahmadu Bello University Teaching Hospital Zaria, Nigeria in 2008. Serum leptin levels by ELISA were determined in 4 groups of individuals including 10 asymptomatic HAART naïve HIV-1 positive patients, 10 symptomatic HAART naïve HIV-1 positive patients, 20 asymptomatic patients on HAART and 26 healthy HIV negative controls. All groups were normal weight adults matched for age and gender. No patient had HIV associated lipodystrophy or abdominal obesity. **RESULTS:** Median leptin levels (and ranges) of asymptomatic patients (45.7ng/ml, 25-60), HAART experienced patients (40.6ng/ml, 7.5-90) and healthy controls (50.2ng/ml, 6.3-110) were not statistically different ( $P>0.05$ ). However, median leptin levels were significantly lower in symptomatic HAART naïve patients (29.9ng/ml (2.8-62.5) when compared to other groups ( $P<0.05$ ). **CONCLUSION:** In normal weight HIV-1 infected patients, effective HAART may regulate serum leptin levels by reversing the leptin suppression that is characteristics of untreated symptomatic HIV infection. The ability of HAART to control the chronic inflammation of untreated HIV and prevent symptomatic disease may underlie this finding.

## INTRODUCTION

Leptin is an adipocyte derived hormone with weight regulatory and immune functions (1, 2). It has been found to depress appetite and promote weight loss in rodents (1) as well as augment cellular immunity in rodent and human studies (2, 3). Serum leptin levels are a direct reflection of the degree of adiposity as leptin levels are higher in obese individuals compared to lean weight individuals while significantly reduced leptin levels have been reported in patients with weight loss and malnutrition (4). Acute inflammatory events such as sepsis and inflammatory products such as tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 1 (IL-1) have been shown to up regulate leptin secretion from adipocytes and consequently increase circulating levels of leptin (5,6). In contrast to this stimulatory role of acute inflammation on leptin, low circulating levels of leptin have been found in studies of normal weight patients with chronic inflammatory disease such as tuberculosis (7) and symptomatic AIDS (8). These low levels have been linked to possible exhaustion and down regulate of leptin secretion by chronic inflammation (7, 8).

Untreated HIV is a chronic inflammatory disease characterised by persistent viral replication, progressive

cellular immunodeficiency, opportunistic infections and weight loss (9-11). Expectedly, leptin levels have been shown to be depressed in patients with HIV associated wasting (12-14) and also in normal weight symptomatic AIDS patients (8). Due to its antiviral and immune-restorative properties, the use of highly active antiretroviral therapy (HAART) prevents opportunistic infections, promotes weight gain and generally reduces the morbidity and mortality associated with HIV/AIDS (9). Some antiretroviral drugs such as the nucleoside transcriptase inhibitors and protease inhibitors may result in subcutaneous fat accumulation and fat redistribution (15) with a secondary increase in circulating leptin levels (16). However, since HAART alleviates the chronic inflammation associated with HIV viral replication and prevents HIV related opportunistic infection, it can be speculated that without regard to fat redistribution or weight gain, leptin levels should be higher in normal weight patients receiving effective HAART when compared to normal weight untreated patients.

There are no studies on serum leptin levels in HAART experienced HIV infected patients from Nigeria. This study was undertaken to determine effect of HAART in regulating serum leptin levels by comparing serum leptin levels in

relation to HAART status in normal weight HIV infected patients.

### **SUBJECTS AND METHODS**

A 3 month cross sectional study was carried out in Ahmadu Bello University Teaching Hospital (ABUTH) in 2008. Ten asymptomatic HAART naïve, 10 symptomatic HAART naïve and 20 asymptomatic HAART experienced HIV-1 infected adults were recruited consecutively as they presented to the medical out patient clinic and the medical wards of ABUTH. All patients were of normal weight and were matched for sex. The HAART naïve patients were newly diagnosed cases of HIV-1 infection. In the HAART experienced group, only asymptomatic patients receiving regular HAART for a minimum of 6 months were included. It is usual that after a minimum of 6 months of effective HAART, HIV viral load becomes undetectable while CD4 cell count stabilizes and increases (17). This is confirmed by our experience in ABUTH HIV treatment centre (unpublished).

Twenty six (13 males, 13 females) HIV negative normal weight healthy adults who were neither hypertensive nor diabetic were recruited to serve as controls. Patients and controls with evidence of abdominal obesity or HAART induced lipodystrophy were excluded.

### **DATA COLLECTION AND CLINICAL EXAMINATION**

Demographic data and clinical history of all study subjects were recorded. Weight (Kg) and height (metres) were measured and body mass index ( $\text{kg/m}^2$ ) was calculated as  $\text{weight}/(\text{height})^2$ . According to the WHO criteria (18) all subjects were classified as normal weight if  $\text{BMI} = 18.5\text{-}24.9\text{kg/m}^2$ .

All patients were clinically examined and evaluated for opportunistic infections. Chest x-ray, Stool, urine and blood culture, lymph node biopsy and other ancillary investigations were done depending on patient's clinical features at presentation.

### **LABORATORY METHODOLOGY**

HIV antibody screening was undertaken using serial rapid test as recommended by the WHO (19). All positive results were confirmed by Western blot (Immunetics Inc, Qualicode HIV1/2 kit, Boston, USA) according to manufacturer's specification. CD4 T cell count was determined by flow cytometry (Partec, GmbH, Munster, Germany) according to manufacturer's specification.

Aprotinin (Trasylol) 200KIU/ml was added to serum samples obtained from all study subjects after which samples were stored at  $-20\text{C}$  until collectively assayed for immunoreactive leptin using an ELISA based assay (Diagnostic Automation Inc, USA, Cat no; 1742-6) according to manufacturer's brochure. Leptin concentrations of serum samples were extrapolated from a standard curve by using the corresponding absorbance of each serum sample. The lowest detectable level of leptin of the assay was  $1\text{ng/ml}$ .

Ethical consideration: Informed consent was obtained from all participants in the study. Approval for the study was obtained from the ABUTH research ethics committee.

### **STATISTICAL ANALYSIS**

Statistical package of social science (SPSS 13) was used for data analysis. Differences between groups were assessed using non-parametric tests.  $P < 0.05$  was considered to be significant.

### **RESULTS**

The median ages and age ranges of symptomatic, asymptomatic, ART experienced and healthy participants were 32yrs (22-54), 35yrs (29-39), 32yrs (26-61), 32yrs (19-60) respectively ( $p > 0.05$ , Kruskal-Wallis test). All participants had normal BMI ranging from  $18.5\text{-}24.9\text{kg/m}^2$ .

### **CLINICAL FINDINGS**

The ART naïve patients were all newly diagnosed cases of HIV-1 infection. The clinical diagnosis of the 10 symptomatic patients included: tuberculosis of the lungs and lymph node ( $n = 3$ ), HIV-related diarrhoea ( $n = 3$ ), typhoid sepsis ( $n = 1$ ), Escherichia coli urinary tract infection ( $n = 1$ ), oral thrush ( $n = 1$ ) and sputum culture negative bronchopneumonia ( $n = 1$ ).

The HAART experienced patients were on first line ART including Lamivudine, Stavudine (or Zidovudine) and Nevirapine (or Efavirenz) for 6 months to 3 yrs with a median duration of 1 yr. No patient was receiving second line protease inhibitor.

### **LEPTIN LEVELS AND CD4 CELL COUNTS IN PATIENTS AND CONTROLS**

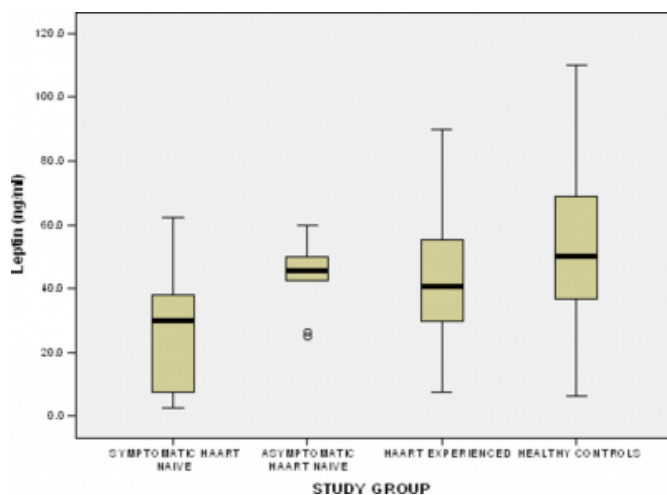
The distribution of serum leptin levels of patient sub-groups and controls are shown in figure 1. The median leptin levels (and ranges) of HAART naïve symptomatic patients ( $29.9\text{ng/ml}$ ,  $2.8\text{-}62.5$ ) was significantly lower than those of HAART naïve asymptomatic patients ( $p = 0.029$ ,  $45.7\text{ng/ml}$ ,

25-60), HAART experienced patients ( $p=0.049$ , 40.6ng/ml, 7.5-90) and healthy controls ( $p=0.003$ , 50.2ng/ml, 6.3-110), Mann Whitney test. Median leptin levels of asymptomatic, HAART experienced and healthy controls were not statistically different ( $P>0.05$  in all groups Mann Whitney test).

The median CD4 cell counts (and ranges in cells/ul) of symptomatic, asymptomatic, HAART experienced and healthy controls were 155 (100-370), 548 (84-1010), 465 (217-901) and 714 (420-1315) respectively ( $P<0.0001$ , Kruskal-Wallis test).

### Figure 1

FIGURE 1: BOX PLOT SHOWING DISTRIBUTION OF SERUM LEPTIN IN PATIENT SUBGROUPS AND IN CONTROLS. NB: Symptomatic patients had significantly lower median leptin level than other groups. The median leptin levels of asymptomatic, HAART experienced patients and healthy controls were not statistically different.



## DISCUSSION

The results of this study are in agreement with a possible down regulation of leptin secretion by the chronic inflammatory response characteristic of untreated symptomatic HIV (8) since significantly lower median leptin levels were found in symptomatic ART naïve patients compared to asymptomatic patients and healthy controls.

Given that the median leptin levels were not significantly different between asymptomatic ART naïve patients, asymptomatic ART experienced patients and healthy controls, it is conceivable that the effect of HAART in sustaining normal circulating leptin levels is related mainly to its ability to prevent symptomatic HIV disease. In support of this assertion, a prospective study of weight stable children revealed that ART increased leptin levels only in

patients receiving effective HAART as leptin levels only increased in patients who had concomitant increase in CD4 T cell count (20). Pagnelli and co-workers (21) have also described concurrent increases in leptin levels and CD4 T cell counts in adult AIDS patients after 2 years on HAART. However, Pinzone and colleagues demonstrated transient rise of leptin levels following initiation of HAART (22) while other workers revealed no change in serum leptin levels after 2 years on HAART (23,24). Although the ART experienced patients in this present study were not followed up by serial prospective measurements of CD4 cell counts and HIV viral load to determine effectiveness of HAART, they were adjudged to be on effective HAART since they were all asymptomatic.

Nucleoside reverse transcriptase inhibitors and protease inhibitors may cause lipodystrophy presenting as abnormal central fat accumulation and or localized fat loss (15). Some studies have shown an increase in serum leptin levels in patients with HIV lipodystrophy (16) while others have not (25). None of the patients in this study developed abdominal obesity or features of HIV related lipodystrophy. Consequently, the reported serum leptin levels in ART experienced patients is unlikely to have been induced by ART related fat deposition or fat redistribution.

Female sex hormones may promote increased or higher leptin levels in females when compared to males of similar BMI, age and nutritional status (1). However, we recruited equal numbers of male and female participants in each group of patients and controls making sex as a confounder of our analysis less likely.

In conclusion, in normal weight HIV-1 infected patients, effective HAART may regulate serum leptin levels by reversing the leptin suppression that is characteristics of untreated symptomatic HIV infection. The ability of HAART to control the chronic inflammation of untreated HIV and prevent symptomatic disease may underlie this finding.

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