METABOLIC MODIFICATIONS IN HIV-INFECTED WOMEN

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To assess metabolic alterations and/or abnormal fat distribution in Human Immunodeficiency Virus (HIV) -infected women undergoing Highly Active Antiretroviral Therapy (HAART), a case-control study was carried out in a population of twenty-two HIV-infected, normal weight, non-diabetic, normotensive women. Twenty-five healthy non infected subjects matched for sex, age and Body Mass Index (BMI) were also included as a control group. Blood samples were collected for leptin and insulin measurements. Fasting glucose, triglycerides, total cholesterol and HDL-cholesterol were also measured. Insulin resistance was determined using the homeostasis model assessment index (HOMA-IR). Body fat distribution was evaluated using waist-to-hip ratio (WHR), Bioelectric Impedance Analysis (BIA) and abdominal CT-scan. Immunologic and virologic parameters included CD4- and CD8-T cell counts and HIV-RNA levels. HIV-infected patients showed higher levels of total cholesterol, LDL-cholesterol and triglycerides ($p<0.05$), higher fasting insulin and HOMA-IR ($p<0.001$), lower levels of HDL-cholesterol ($p<0.001$) and serum leptin ($p<0.001$) than the control group. With regard to body fat distribution, no statistically significant difference between cases and controls was found. Among the control women leptin levels were positively correlated with body fat distribution parameters ($p<0.001$).

The benefits of highly active antiretroviral therapy (HAART) are compromised by a lot of side effects including peripheral fat wasting, abnormal fat distribution, hyperlipidemia, and insulin resistance (1-2).

Lipodystrophy is a major side-effect of antiretroviral therapy whose pathophysiology remains unclear. In vitro studies show that Human Immunodeficiency Virus type 1 (HIV-1) -protease inhibitors affect adipocyte differentiation at an early step involving sterol regulatory element binding protein-1, but in vivo studies are lacking (3).

However, insulin resistance and dyslipidemia can occur in HIV- infected patients with or without lipodystrophy. Protease inhibitors could play an important role in the development of metabolic disorders independently of HIV status (4).

HIV protease inhibitors suppress protease-mediated breakdown of nascent apolipoprotein B thus resulting in the overproduction and secretion of triglyceride-rich lipoproteins (1).

Possible mechanisms whereby protease inhibitors can hinder insulin actions include inhibition of glucose transporter isoform Glut 4, and altered expression of leptin in adipose tissue (4).

Leptin is an adipocyte-specific ob gene product (5) which has been involved in the pathophysiology of human obesity (6-7) and other clinical states.

Key words: human immunodeficiency virus, antiretroviral therapy, metabolic modifications, leptin, women

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Women show significantly higher leptin levels than men (8-9); nevertheless, differences in serum leptin levels between central and peripheral fat distribution in obese subjects have not been clearly demonstrated yet and contrasting findings are available on the relationship between fat distribution and leptin levels (10-12).

The aim of this work is to assess metabolic alterations with particular regard to leptin levels in women with HIV infection undergoing HAART therapy.

MATERIALS AND METHODS

Patients

Twenty-two HIV-infected Caucasian women, aged 37.7 ± 4.7 (SD) years, were enrolled at the first visit at the Infectious Diseases Division of Chieti University. These patients had received ≥ 12 months (median: 47; range: 107) of cumulative exposure to antiretroviral regimen, which included teraphy with Protease Inhibitors (PIs) and/or total Nucleoside Reverse-Transcriptase Inhibitor (NRTi) and/or Non Nucleoside Reverse Transcriptase Inhibitor (NNRTi). They were normal weight [Body Mass Index – BMI – (calculated as weight in kilograms devided by the square of height in meters) 24.1 ± 5.4], they had a normal glucose tolerance test (13), and normal blood pressure. Twenty-five non-obese uninfected healthy subjects, matched for sex, ethnicity, age and BMI (age 37.5 ± 4.7 years, BMI 22.8 ± 1.8 kg/m^2) were also included as a control group.

The study was approved by the Medical Ethics Committee of the "G. d'Annunzio" University Medical School. All women gave written informed consent.

Anthropometric and fat distribution indexes

Anthropometric dimensions were taken at morning by a trained staff according to WHO recommendations (14). Height, weight and waist and hip circumferences were measured while the subjects wore indore clothes without shoes. Body mass index and WHR were computed. The WHR was defined as the minimal abdominal circumference between the xiphoid process and the iliac crests (waist) divided by the circumferences determined over the femoral heads (hip). The WHR cutoff point used to discriminate between abdominal and gluteal fat distribution was 0.86 (abdominal or android type WHR ≥ 0.86; peripheral or gynoid type WHR < 0.86) (15).

Bioelectric Impedance Analysis (BIA) was used to evaluate lean body mass, total body water, body cell mass and fat body mass (16).

A CT-scan was performed at L4-L5 level to calculate total area of abdominal, visceral and subcutaneous fat (17).

Blood measurements

CD4- and CD8-T cell counts were obtained by cytofluorimetric assessment of lymphocyte subpopulations.

Plasma viral load (HIV-RNA) was determined using the "Amplicor" method (Roche Mol. Dagn. Milan, It.), with detection limit ≤ 400 HIV RNA copies/mL plasma.

Fasting blood samples were drawn to measure glucose, leptin, insulin, and lipid levels. Fasting glucose was measured by glucose-oxidase method.

Serum leptin concentrations were calculated by an enzyme-linked immunosorbent assay (Leptin ELISA - DRG Instruments GmbH - Germany). The limit of detection was 0.2 ng/mL, and the intra- and inter- assay coefficients of variation were 4.6% and 6.6%, respectively.

Insulin levels were measured by RIA (Coat-A-Count Insulin kit, Diagnostic Products Corporation, Los Angeles, USA). The sensitivity and the intra- and inter-assay coefficients of variation were 1.2 μU/mL, 4.7% and 7.3%, respectively.

Insulin resistance was determined using the homeostasis model assessment index (HOMA-IR) (18).

Triglycerides, total cholesterol and high density lipoprotein (HDL) cholesterol were determined by an automated enzymatic method (Ortho-Clinical Diagnostics – Rochester, NY, USA).

LDL-cholesterol was calculated using Friedewald's formula (19).

Statistics

Data are presented as mean ± standard deviation (SD). Statistical significance was assessed by Student's *t* test. A P value of < 0.05 was required for statistical significance. Pearson's correlation coefficients were computed between serum leptin levels and fat distribution parameters.

RESULTS

Subjects with HIV infection and controls were matched for sex, age and BMI.

No statistically significant differences between

Tab. I. *Clinical and anthropometric characteristics (mean ± SD).*

	HIV patients (n. 22)	**Controls** (n. 25)
Sex	F	F
Age (years)	37.77 ± 4.79	37.56 ± 4.70
SBP (mmHg)[1]	117.09 ± 8.84	113.60 ± 10.36
DBP (mmHg)[1]	70.95 ± 5.44	69.72 ± 5.11
BMI (Kg/m^2)[2]	24.11 ± 5.45	22.83 ± 1.81
WHR[3]	0.81 ± 0.08	0.77 ± 0.06
Fat mass (%)	24.31 ± 7.36	22.36 ± 5.04
Free fat mass (%)	74.45 ± 6.50	76.32 ± 5.68
Total AAT (cm^2)[4]	198.31 ± 47.31	195.16 ± 43.40
Subcutaneous AAT (cm^2)[4]	135.99 ± 32.25	137.12 ± 38.41
Visceral AAT (cm^2)[4]	62.31 ± 27.05	57.44 ± 6.64

[1] *SBP, DBP: Systolic and Diastolic Blood Pressure;*
[2] *BMI: Body Mass Index;*
[3] *WHR: Waist-to-Hip Ratio;*
[4] *Abdominal Adipose Tissue.*

Tab. II. *HIV infection parameters (mean ± SD).*

	HIV patients (n. 22)		**Controls** (n. 25)
CD4 Tcell counts	564.86 – 306.10	P < 0.001	1134.67 – 193.49
CD8 Tcell counts	803.50 – 427.72		779.33 – 208.54
HIV-RNA (copies/mL)	4951.43 – 3220.49		undosable

Tab. III. *Metabolic parameters (mean ± SD)*

	HIV patients (n. 22)		**Controls** (n. 25)
Total cholesterol (mg%)	194.09 ± 70.45	P < 0.05	159.92 ± 10.76
HDL cholesterol (mg%)	48.00 ± 5.83	P < 0.001	57.56 ± 7.25
Triglycerides (mg%)	163.72 ± 185.76	P < 0.05	82.84 ± 29.69
LDL cholesterol (mg%)[1]	118.30 ± 63.70	P < 0.05	79.79 ± 16.92
Fasting glucose (mmol/L)	4.60 ± 0.52		4.75 ± 0.37
Fasting insulin(mcU/mL)	40.22 ± 9.91	P < 0.001	10.39 ± 3.37
HOMA-IR[2]	8.17 ± 2.06	P < 0.001	2.18 ± 0.73
Leptin (ng/mL)	2.61 ± 1.06	P < 0.001	9.39 ± 4.36

[1] *LDL-cholesterol: calculated using the Friedewald's formula;*
[2] *HOMA-IR: Homeostasis Model Assessment index for Insulin Resistance.*

Tab. IV. *Pearson's correlation coefficients (r) of leptin levels with adipose tissue parameters among control women.*

Fat mass	r = 0.91	P < 0.001
Free fat mass	r = 0.93	P < 0.001
Total Abdominal Adipose Tissue	r = 0.89	P < 0.001
Subcutaneous Abdominal Adipose Tissue	r = 0.86	P < 0.001
Visceral Abdominal Adipose Tissue	r = 0.85	P < 0.001

cases and controls were documented for body fat distribution (WHR, Bioelectric Impedance Analysis, TC-scan) (Tab. I).

HIV-infected women showed lower CD4-T cell count than control women, while CD8-T cell count wasn't significantly different between cases and controls. The levels of HIV-RNA in HIV infected women were 4951.43 ± 3220.4 copies/ mL (Tab. II).

Patients with HIV, when compared with the control group, showed: 1) higher blood levels of total cholesterol, LDL-cholesterol and triglycerides ($p<0.05$); 2) lower levels of HDL-cholesterol ($p<0.001$); 3) higher serum levels of fasting insulin (40.22 ± 9.91 *versus* 10.39 ± 3.37) and HOMA-IR (8.17 ± 2.06 *versus* 2.18 ± 0.73 – $p<0.001$); 4) lower levels of serum leptin (2.61 ± 1.06 *versus* 9.39 ± 4.36 – $p<0.001$) (Tab. III). Among control women leptin levels positively correlated with body fat distribution parameters ($p<0.001$) (Tab. IV).

DISCUSSION

In the present study, we documented significant metabolic alterations in patients with HIV infection. In our HIV-infected patients the side effects of highly antiretroviral therapy principally were the decrease of leptin levels, insulin resistance and hyperlipidemia. These modifications probably lead to the lipodystrophic damage well documented in literature (20). Previous studies evaluating leptin levels in patients with HIV-related lipodystrophy have reported conflicting data (21). Our results agree with both recent studies (3, 22) which demonstrated lower leptin levels in patients with HIV than in controls and with Yarasheski and Paganelli (23-24) who documented no relationship between leptin levels and lipodystrophy. On the contrary, Kosmiski et al.(25) showed significantly higher serum leptin levels in HIV patients with lipodistrophy and Mynarcik (26) documented similar leptin levels in HIV-infected patients and controls. In our study no HIV-infected woman had pathological BMI (obesity or wasting syndrome) and/or pathological body fat distribution.

This is why, we did not find any association between HIV-infected women serum leptin levels and insulin resistance and/or body fat distribution: in other studies these correlations were found in patients with lipoatrophy and/or with lipohypertrophy (21).

In particular, leptin levels were lower in patients with lipoatrophy and higher in patients with lipohypertrophy (21, 27).

Leptin decrease reported in our patients may be the result of previous fat loss leading to decreased synthesis and release of leptin from adypocytes (21).

According to literature data, HIV-uninfected subjects plasma leptin levels positively correlated with central fat accumulation and they were associated with insulin resitance (7, 11).

Furthermore, as in obese subjects, in HIV-infected patients we documented an insulin resistance condition which could be related to an inflammatory status, as demonstrated by an increase of interleukin-6 and tumor necrosis factor (28).

In addition, our results confirm lipid abnormalities (including elevated total cholesterol, triglycerides and LDL-cholesterol levels and decreased HDL-cholesterol levels), documented in up to 60% of patients treated with protease inhibitors (29-32).

In conclusion, plasmatic leptin levels could be good predictor of HIV-related metabolic alterations but not of body fat redistributions (33).

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