

# Effects of Viral Replication on Immune Activation and Fat Metabolism in a Cohort of Treatment-naïve HIV-1-infected African Women.

Livio Azzoni<sup>1</sup>, Nigel Crowther<sup>2</sup>, Cynthia Firnhaber<sup>2</sup>, Andrea Foulkes<sup>3</sup>, Deborah Glencross<sup>2</sup>, Mitch Kaplan<sup>4</sup>, Wendy Stevens<sup>2</sup>, Ian Sanne<sup>2</sup>, Tessa van der Merwe<sup>5</sup> and Luis J. Montaner<sup>1</sup>

1 The Wistar Institute, Philadelphia, PA, USA; 2 University of the Witwatersrand, Johannesburg, South Africa; 3 University of Massachusetts, Amherst, USA; 4 Rosebank Clinic, Johannesburg, South Africa; 5 University Pretoria and Network Healthcare Holdings, Pretoria, South Africa

**Introduction:** Progression of HIV infection results in fat loss and wasting. Leptin is a hormone produced by adipose tissue and contributes to the regulation of caloric intake and fat accumulation, as well as modulating lymphocyte responses; advanced HIV infection has been shown to result in fat loss and low serum leptin levels; however, leptin levels have been reported to be normal even in advanced HIV infection in the absence of secondary infections. While prior studies (e.g. FRAM) have addressed the effects of antiretroviral therapy (ART) and ART interruption on fat metabolism, the direct effects of HIV infection on fat metabolism, particularly in women, remain unclear. This study assesses the effect of HIV replication levels on fat accumulation and leptin levels in a population of ART-naïve HIV-1-infected South African women with no history of opportunistic infections.

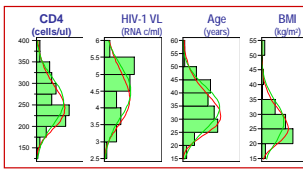
**Summary of conclusions**  
We have assessed immunologic and metabolic parameters in a cohort of 85 women initiating anti-retroviral treatment at the CHRU clinic in Johannesburg (South Africa). Our data indicate that:

- HIV-1 infected women with higher viral load have significantly lower fat accumulation (and lean mass, not shown) than those with lower VL.
- HIV replication level is directly correlated to body fat depletion.
- Women with different levels of HIV replication had similar levels of insulin resistance markers, bone density measures, LFTs and other parameters studied.
- Lipolysis is unlikely to be the primary cause of body fat loss.
- As observed in healthy individuals, leptin levels are directly correlated with body fat accumulation.
- Leptin levels are negatively associated with viral load; this is maintained after adjustment for BMI, suggesting and independent association.
- HIV replication is an independent predictor of leptin levels in this cohort.

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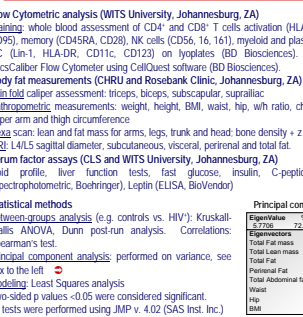
**Abstract**  
**Background:** Progression of HIV infection results in fat loss and wasting. However, leptin levels have been reported to be normal in advanced HIV infection in the absence of secondary infections, and repeated ART interruptions may increase leptin levels. We investigated the direct effects of HIV replication on fat metabolism, particularly in women, remain unclear.  
**Methods:** Study subjects: 85 HIV-1-infected, ART-naïve women (median CD4 T-cell count: 424 cells/μL, median log<sub>10</sub> VL: 4.78 copies/mL, no prior ID). Controls: 50 healthy female volunteers. Study location: Johannesburg, RSA. Informed consent was obtained from all subjects. Fresh blood samples were obtained on pre-mixed silica lyophilized on 96-well plates, and analyzed by flow cytometry (BD Biosciences).  
**54 metabolic variables** were collected. Total, LDL, HDL, HDL cholesterol, triglycerides, leptin, free fatty acids (FFA), insulin and C-peptide, fasting glucose were assayed on fresh or cryopreserved serum. Body fat distribution was assessed by DEXA scan (with bone density), MRI (4 L4-L5 cross section) and anthropometric measurements. BMI was calculated.  
**Statistical analysis:** Between-group differences/comparisons (Spearman test, Kruskal-Wallis ANOVA), Principal Component on variance analysis, Standard Least Squares analysis.  
**Results:** As expected, VL was negatively correlated to CD4 counts and positively correlated to CD206/HLA-DR expression on T cells. Plasmacytoid dendritic cells (but not myeloid DC or NK cellular) were more in women with VL < median. Both leptin and all body fat measurements, but not bone density, were negatively correlated to VL. Accordingly, no significant differences in leptin and fat accumulation was observed in women with VL < median, as compared to control or low VL HIV-infected women. No difference was observed in FFA or serum insulin, indicating the absence of differential lipolysis. Leptin levels were positively correlated to fat levels in all subjects. In HIV-infected women the leptin/BMI ratio remained negatively correlated to VL, suggesting an independent effect of viral replication on leptin levels. We further explored this, eventually using a standard least square model using log<sub>10</sub> VL and total fat (assessed by MRI) as predictors; the results indicate that VL is an independent predictor of serum leptin levels.  
**Conclusions:** HIV-1 replication, associated with fat loss, immune activation and depletion of adaptive and innate immunity effectors, is an independent predictor of serum leptin levels in women.

**Methods**  
**Cohort description:** 85 HIV-1 infected women attending the CHRU clinic at Helen Joseph Hospital, Johannesburg, RSA. 50 healthy women volunteer recruited at Johannesburg General Hospital, Johannesburg, RSA.

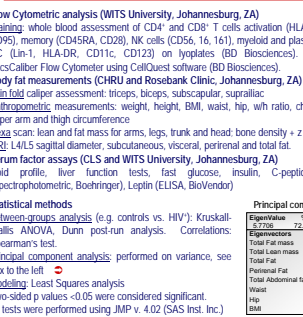


**Flow Cytometric analysis** (MITS University, Johannesburg, ZA)  
**Staining:** whole blood assessment of CD4<sup>+</sup> and CD8<sup>+</sup> T cells activation (HLA-DR and CD95), memory (CD45RA, CD28), NK cells (CD56, 16, 161), myeloid and plasmacytoid DC (Lin-1, HLA-DR, CD11c, CD123) on lyoplates (BD Biosciences).  
**Analysis:** FACScan Flow Cytometer using CellQuest software (BD Biosciences).  
**Body fat measurements** (CHRU and Rosebank Clinic, Johannesburg, ZA)  
**Skin fold caliper assessment:** triceps, biceps, subscapular, suprailiac.  
**Anthropometric measurements:** weight, height, BMI, waist, hip ratio, chest, mid-upper arm and thigh circumference.  
**DEXA scan:** lean and fat mass for arms, legs, trunk and head: bone density + z ratio  
**MRI:** L4/L5 sagittal diameter, subcutaneous, visceral, perirenal and total fat.  
**Serum factor assays** (CLS and WITS University, Johannesburg, ZA)  
**Lipid profile:** liver function tests, fast glucose, insulin, C-peptide, FFA (Spectrophotometric, Biohinger), Leptin (ELISA, BioVendor)

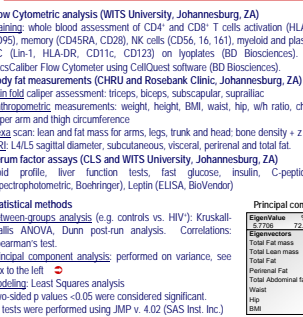
**Statistical methods**  
**Between-groups analysis** (e.g. controls vs. HIV-1): Kruskal-Wallis ANOVA, Dunn post-hum. correlations.  
**Spearman's test.**  
**Principal component analysis** performed on variance, see box to the left.  
**Least Squares analysis**  
**Modeling:** Least Squares analysis  
Two-sided p values < 0.05 were considered significant.  
All tests were performed using JMP v. 4.02 (SAS Inst. Inc.)



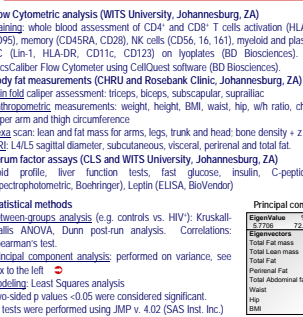
**2 Body fat is depleted in women with high levels of viral replication, and HIV-1 viral load is inversely correlated to body fat accumulation**



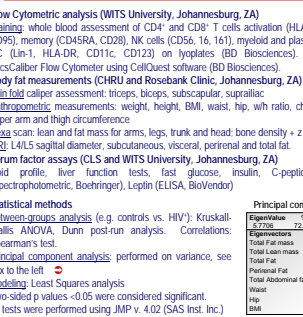
**3 Leptin levels are strongly correlated with fat accumulation; an independent effect of VL on leptin levels is suggested by the persistence of the negative correlation after adjustment for BMI**



**4 High viral replication is associated with low leptin levels, but not increased free fatty acids (lipolysis is not the primary cause of fat loss)**



**5 HIV-1 viral load is an independent predictor of serum leptin levels in HIV-infected South African women**



**6 Summary of findings**

Characteristic	Mean (SD)	Median (IQR)
Age (years)	37.0 (10.0)	36.0 (28.0-44.0)
Weight (kg)	60.0 (15.0)	58.0 (48.0-68.0)
Height (cm)	160.0 (6.0)	159.0 (153.0-167.0)
BMI (kg/m <sup>2</sup> )	23.0 (4.0)	22.0 (19.0-26.0)
Waist (cm)	85.0 (12.0)	83.0 (75.0-93.0)
Hip (cm)	95.0 (10.0)	93.0 (85.0-101.0)
Waist:hip ratio	0.88 (0.04)	0.87 (0.81-0.93)
Mid-upper arm (cm)	25.0 (2.0)	24.0 (22.0-26.0)
Thigh (cm)	35.0 (3.0)	34.0 (31.0-37.0)
Leptin (ng/ml)	15.0 (10.0)	12.0 (8.0-18.0)
Free fatty acids (mmol/L)	0.45 (0.10)	0.42 (0.35-0.50)
Triglycerides (mmol/L)	1.50 (0.50)	1.20 (0.80-1.80)
Total cholesterol (mmol/L)	4.50 (1.00)	4.20 (3.50-5.00)
HDL cholesterol (mmol/L)	1.20 (0.30)	1.10 (0.80-1.50)
LDL cholesterol (mmol/L)	2.80 (0.80)	2.60 (2.00-3.40)
Fasting glucose (mmol/L)	5.00 (0.50)	4.80 (4.50-5.30)
Insulin (mU/L)	10.00 (3.00)	9.00 (7.00-12.00)
C-peptide (ng/mL)	0.80 (0.20)	0.75 (0.60-0.90)
T cell memory subsets (%)	15.0 (5.0)	14.0 (10.0-18.0)
NK cell subsets (%)	10.0 (3.0)	9.0 (7.0-12.0)

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