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CYTOKINE PROFILE AND CLINICAL METABOLIC ALTERATIONS IN HIV-1 INFECTED INDIVIDUALS WITH AND WITHOUT LIPODISTROPHY

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ABSTRACT: The extensive use of Highly Active Antiretroviral Therapy (HAART) has transformed HIV infection into a chronic condition. Thus, metabolic alterations including lipodystrophy and dyslipidemia have been associated with the use of such medications. The objective of the present study was to analyze clinical metabolic alterations and the profile of TNF-α, IFN-γ, IL-2, IL-10, and TNF-α type II soluble receptor in serum of HIV-1 individuals with and without lipodystrophy. Eighty-four adults were evaluated, 42 males and 42 females, mean age 37 years, and HAART time of at least 15 months. Two groups were formed, G1: 42 individuals with lipodystrophy, and G2: 42 without lipodistropy. From the HAART used, stavudine was more associated with the lipodystrophy group and zidovudine with the non-lipodystrophy group. CD4 and CD8 values, viral load, glucose, albumin, and lipids were not different between groups, except for triglycerides, which were high in the lipodystrophy group, and HDL, whose concentration was reduced in G1. TNF-α, TNF-RII, and IL-10 profiles were high and had positive correlation; IL-2 and IFN-γ had reduced levels in the lipodystrophy group. High TNF-α and its receptor levels seem to be associated with lipodystrophy development in individuals under HAART therapy.

KEY WORDS: lipodystrophy, HIV-1, hypertriglyceridemia, cytokines.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

The extensive use of potent combined antiretroviral therapy (HAART) has lead to an important reduction in morbidity and mortality associated with the HIV virus, transforming this disease into a chronic condition (35). Therefore, changes in prognosis, quality of life, and survival of many patients have been observed (30,35).

Changes in the nutritional status associated with the use of these new therapies have also been observed in association with the patient's longer survival (6). Alterations in glucose, triglycerides and cholesterol serum levels, other metabolic alterations, and body fat redistribution (lipodystrophy) have been reported (2,5,6,36) and lipid metabolism has been associated with cytokines which are produced during infections (20).

Biochemical alterations and participation of the immune system in lipodystrophy pathogenesis are still unknown. However, reports have been made in an attempt to explain these alterations and their possible involvement with antiretroviral therapy and the immune system (5, 6, 11, 17, 25, 26). Several authors have reported Protease Inhibitors (PI) with cytokines such as TNF-α, IFN-α, IFN-γ, IL-1, IL-6, and IL-12, in the involvement in both immune reconstitution and lipid manipulation (5, 6, 10, 11,25, 26). In addition, the HIV PI immunomodulatory effect cannot be excluded in T cells. Such drugs suppress physiological apoptosis by inhibiting cellular proteases (39) and have a direct impact on cytokine production (1).

Ledru *et al*. (25) showed that the PI direct effect on cell proteases also have to be considered as a mechanism contributing to the accumulation of T cells, producers of TNF-α. Therefore, such polarization of T cells to synthesize TNF-α seems to favor lipodystrophy by contributing to changes in lipid metabolism. In fact, significant positive correlations were seen between the absolute number of CD8 T cells, production of TNF-α, and lipid parameters generally altered in lipodystrophy including cholesterol, triglycerides and the B/apo A1 alipoprotein atherogenic ratio (25). In another study, the same authors (26) did not see increased Th2 cytokine profile in HIV infected patients, even those under HAART therapy, which suggests the role of apoptosis in TNF-α physiological regulation and negative regulation by T cells.

Based on previous considerations, it is extremely important to study the interaction between nutrition, infection, and immunity in AIDS, especially the production of TNF-α, IFN-γ, IL-2, IL-10 and TNF-α type II soluble receptor (sTNF-RII), in serum

produced by HIV-1 infected individuals with or without lipodystrophy by analyzing the patients clinical metabolic characteristics and possible association with lipodystrophy. Such studies improve knowledge on these very complex aspects and certainly contribute to a better approach in managing this disease.

PATIENTS AND METHODS

Patients

Between January 2003 and September 2004, 84 adults with confirmed HIV infection were analyzed, 42 (50%) were female, and 42 (50%) male, mean age was 37 years; they were under HAART for 15 or more months. They were seen at the Special Infectious Diseases Outpatient Clinic, Botucatu School of Medicine, UNESP.

Excluded from the study were those with severe acute diseases or on steroids, anabolic steroids, glucocorticoids, immunomodulatory drugs or other drugs which could interfere with their metabolism, and those under 18 or pregnant.

Individuals were divided into the following two groups: G1 – 42 individuals of both sexes, with HIV-1 and lipodystrophy; G2 – 42 individuals of both sexes, with HIV-1 and without lipodystrophy.

This study was approved by the Ethics Committee of Botucatu Medical School, and informed consent was obtained from all patients.

Methods

Diagnosis of Lipodystrophy Syndrome: Individuals with lipodystrophy were those who showed clinical evidence of at least two of the following morphological alterations, adapted from Carr *et al*., (7) loss of fat from the face, arms, legs, or buttocks (with or without prominent veins in legs or arms); fat accumulation in the abdomen, breast or dorsocervical region. These patients might also have dyslipidemia. The clinical factors were identified by both the doctor and the patient, and both needed to agree with fat accumulation or loss for the patient to be included in G1.

Clinical Epidemiological Aspects: The following clinical epidemiological aspects were considered: age, smoking (>5 cigarettes/day), sedentariness (<1h/week physical exercise), alcoholic beverages (>80g/day), weight loss (>5% total body

weight), diarrhea (>2 liquid evacuations/>2 days), any fever episode, anorexia, and nausea in the last two months. Evaluation of body composition included measurement of weight/height^2 (kg/m^2) to obtain Body Mass Index (BMI).

Laboratory Investigation: Biochemical and immunological examinations were performed. Peripheral blood samples were obtained through venous puncture in the morning after 8–12 hours fasting.

Biochemical Examination: Serum glucose (mg%) was determined using the colorimetric enzymatic method (GOD-PAP), Biotrol Diagnostic Kit, and albumin (g%) was determined using the Bromo Cresol Green colorimetric method, Proti Kit, Wiener Lab., in a model RA-XT Technicon Auto-analyzer. Serum total cholesterol (mg%) was determined using enzymatic method, Flex® Cholesterol Kit, DADE BEHRING, and triglycerides (mg%) were deteremined using enzymatic method, Flex® Triglycerides Kit, DADE BEHRING in a DADE BEHRING Dimension® Clinical Chemistry System. Serum HDL, LDL, VLDL were determined using gel electrophoresis in a REP Electrophoresis apparatus, Helena BioSciences Europe.

Immunological Examination: To evaluate immunological activity, we used the quantitative determination of T lymphocyte sub-population (cells/mm^3) with CD_4^+ and CD_8^+ markers through flow cytometry using a Cyto-Stat®/Coulter Clone® CD3 (IgG1)-FIT/T4-RD1 and Cyto-Stat®/Coulter Clone® CD3 (IgG1)-FIT/T8-RD1. For plasmatic viral load (number of copies/ml), Nuclisens™ HIV-1/QT NASBA DIAGNOSTICS-ORGANON TEKNIKA kit was used.

Serum Cytokine Determination: Serum levels of TNF-α, IFN-γ, IL-2, IL-10, and sTNF-RII were determined through ELISA, using Quantikine R&D Systems kits, Minneapolis, MN, USA, according to the manufacturer's instructions. Sensitivity limit for all parameters was 10pg/ml.

Statistical Analysis: Comparisons between groups, expressed in proportion of occurrences per group (G1 and G2), were performed using the χ^2 test or Fisher's exact method. Mean and standard deviations were calculated for quantitative

variables in each group and comparisons between groups were made using the Student's t test for two independent samples. Correlation between pairs of quantitative variables was by linear coefficient of correlation in each group separately. In all analyses, significance was $p<0.05$ (14).

RESULTS

Comparison between G1 and G2 for gender, sexual transmission mechanism, and clinical epidemiological parameters such as smoking, fever, diarrhea, weight loss, and anorexia was not statistically different; however, G1 had fewer alcohol drinker and sedentary individuals than G2 ($p<0.05$). There was also no statistical difference between groups for age and time of the last therapy. Duration of HIV-1 infection and total antiretroviral therapy were both significantly longer in G1 (99.4±43.0 and 78.5±29.5 months) than in G2 (71.4±35.6 and 58.6±24.8 months), respectively ($p<0.05$).

For antiretroviral drugs and regimen, statistical difference was seen between the groups for number of patients under treatment for at least 15 months with zidovudine (AZT) (G1<G2) and stavudine (d4T) (G1>G2), but there was no difference between groups in relation to the antiretroviral class ($p>0.05$; Table 1).

Analysis of albumin, glucose, total and LDL cholesterol, T lymphocyte count with CD_4^+ and CD_8^+ markers, BMI, and viral load showed no significant difference between groups (Table 2). Only G1 triglyceride serum levels were higher than that of G2 ($p<0.05$). Reduced or normal HDL levels, considering gender, revealed more G1 patients with lower than normal values, compared with G2 ($p<0.05$; Table 3).

G1 cytokine serum levels of TNF-α, TNF Receptor II, and IL-10 were significantly higher, and IL-2 and IFN-γ levels were significantly lower than those of G2 ($p<0.05$; Table 4).

Tables 5 and 6 show no association between lipid profile and cytokines between groups. However, there was correlation between cytokines and lipid profile within each group; G1 had positive correlation between IL-10, TNF-α, and sTNF R-II, and between TNF-α and sTNF R-II; and negative correlation between TNF-α and IFN-γ, and between IFN-γ and sTNF R-II.

Table 6 shows G2 with the same correlations and significant correlation between IL-10 and IL-2, and between IL-10 and INF-γ.

Table 1. Antiretroviral drug and regimen distribution of 83* HIV-1 infected individuals, according to groups.

Parameters	**G1 (n=41*)**		**G2 (n=42)**		**Significance**	**Comments**
	n	**(%)**	**n**	**(%)**		
Drugs						
AZT	19	45	34	81	p<0.01	G1<G2
3TC	32	76	35	83	p>0.50	G1=G2
d4T	21	50	8	19	p<0.02	G1>G2
ddI	8	19	7	17	p>0.50	G1=G2
IDV	13	31	6	14	p>0.05	G1=G2
RTV	7	17	2	5	p>0.05	G1=G2
NFV	5	12	9	21	p>0.10	G1=G2
NVP	5	12	10	24	p>0.10	G1=G2
EFV	8	19	9	21	p>0.5	G1=G2
Anti-retroviral regimen						
PI + NRTI	23	27.7	20	24.1	p>0.50	G1=G2
RTINN + NRTI	15	18.1	19	22.9	p>0.50	G1=G2
Others	3[+]	3.6	3[#]	3.6	p>0.50	G1=G2

G1: HIV-1[+] individuals with lipodystrophy; G2: HIV-1[+] individuals without lipodystrophy.

*: It was not possible to discriminate the antiretroviral therapy of one patient

[+]: One patient on antiretroviral therapy only with analogues, and two patients on antiretroviral therapy with nucleotide analogue, non-analogue and protease inhibitor in G1

[#]: Three patients on antiretroviral therapy only with analogues in G2.

AZT: zidovudine; 3TC: lamivudine; d4T: stavudine; ddI: didanosine; IDV: indinavir; RTV: ritonavir; NFV: nelfinavir; NVP: nevirapine; EFV: efavirenz; PI: protease inhibitor; NRTI: nucleotide reverse transcriptase inhibitors; NNTRI: non-nucleoside reverse transcriptase inhibitors.

Table 2. Mean ($\overline{x}$) and Standard Deviation (SD) of biochemical parameters, CD4 and CD8 T lymphocyte counts and body mass index of 84 HIV-1 infected individuals, according to groups.

Serological Exams	G1		G2		Statistics	Comments
	$\overline{x}$	SD	$\overline{x}$	SD		
Albumin (g%)	4.49	0.38	4.52	0.41	t=0.349; p>0.50	G1=G2
Glucose (mg%)	100.69	57.41	90.38	8.92	t=1.150; p>0.10	G1=G2
Total Cholesterol (mg%)	165.74	35.62	167.14	33.44	t=0.186; p>0.50	G1=G2
Triglycerides (mg%)	266.71	168.34	167.80	109.91	t=3.188; p<0.01	G1>G2
LDL (mg%)	114.87	28.89	118.01	29.31	t=0.494; p>0.50	G1=G2
BMI (kg/m^2)	23.29	3.30	23.77	3.34	t=0.663; p>0.50	G1=G2
CD4 ($cell/mm^3$)	412.21	232.57	383.88	170.80	t=0.636; p>0.50	G1=G2
CD8 ($cell/mm^3$)	1067.38	442.86	1018.95	383.65	t=0.536; p>0.50	G1=G2
CD4/CD8	0.405	0.242	0.408	0.201	t=0.061; p>0.50	G1=G2
Viral load (log of copies/ml)	3.01	1.14	2.95	1.08	t=0.248; p>0.50	G1=G2

G1: HIV-1^+ individuals with lipodystrophy; G2: HIV-1^+ individuals without lipodystrophy.

LDL: low density lipoprotein; VLDL: very low density cholesterol; BMI: body mass index, Log: logarithm of number of RNA copies of HIV/ml.

Table 3. Distribution of 84 HIV-1 infected individuals, according to groups, and classification of HDL level into normal or reduced in relation to gender.

HDL mg%	**G1 (n=42)**		**G2 (n=42)**		**Total (n=84)**		**Significance**	**Comments**
	n	**(%)**	**n**	**(%)**	**n**	**(%)**		
Normal	26	62	37	88	63	75	p<0.05	**G1=G2**
Reduced	16	38	5	12	21	25	p<0.05	**G1>G2**

G1: HIV-1$^+$ individuals with lipodystrophy; G2: HIV-1$^+$ individuals without lipodystrophy.

Table 4. Mean ($\bar{x}$) and standard deviation (SD) of serum cytokines in 84 HIV-1 infected individuals, according to groups.

Parameter	**G1 (n=42)**	**G2 (n=42)**	**Significance**	**Comments**
TNF-α (pico/μl)	635.5±131.9	351.8±146.9	p<0.001	G1>G2
TNF-α Receptor II (pico/μl)	820.4±126.5	539.0±148.1	p<0.001	G1>G2
Interleukin-10 (pico/μl)	49.2±12.6	39.2±16.5	p<0.01	G1>G2
Interleukin-2 (pico/μl)	101.8±23.5	113.3±23.0	p<0.02	G1<G2
IFN-γ (pico/μl)	361.1±149.6	568.5±174.3	p<0.001	G1<G2

G1: HIV-1$^+$ individuals with lipodystrophy; G2: HIV-1$^+$ individuals without lipodystrophy.

Table 5. Correlations between lipid profile and cytokines in G1 (with lipodystrophy).

Correlation G1	Receptor TNFII	IL-10	IL-2	INF-γ	TNF-α	Cholesterol	LDL	HDL	VLDL	Triglycerides
Receptor TNFII	-	0.36*	-0.22	-0.34*	0.92**	0.15	0.16	0.08	-0.26	-0,11
IL-10	-	-	0.27	-0.22	0.36*	0.11	0.20	-0.067	-0.08	0.09
IL-2	-	-	-	-0.05	-0.25	0.02	0.02	0.02	0.13	0.04
INF-γ	-	-	-	-	-0.36*	-0.22	-0.17	-0.07	0.01	-0.24
TNF-	-	-	-	-	-	0.18	0.20	0.09	-0.29	0.10
Cholesterol	-	-	-	-	-	-	0.94**	0.63**	-0.15	0.06
LDL	-	-	-	-	-	-	-	0.46**	-0.17	-0.03
HDL	-	-	-	-	-	-	-	-	-0.45**	-0.42**
VLDL	-	-	-	-	-	-	-	-	-	0.65**
Triglycerides	-	-	-	-	-	-	-	-	-	-

G1: HIV-1^+ individuals with lipodystrophy.

HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density cholesterol.

*: $p<0.05$; **: $p<0.01$.

Table 6. Correlations between lipid profile and cytokines in G2 (without lipodystrophy).

Correlation G2	Receptor TNFII	IL-10	IL-2	INF-γ	TNF-α	Cholesterol	LDL	HDL	VLDL	Triglycerides
Receptor TNFII	-	0.69**	-0.25	-0.44**	0.90**	-0.05	-0.15	0.03	0.16	-0.02
IL-10	-	-	-0.52**	-0.43**	0.70**	0.04	-0.10	0.10	0.16	0.08
IL-2	-	-	-	0.30	-0.21	-0.14	0.05	-0.22	0.03	0.16
INF-γ	-	-	-	-	-0.46**	-0.04	0.15	-0.23	0.05	0.12
TNF-α	-	-	-	-	-	0.02	-0.06	0.03	0.09	-0.02
Cholesterol	-	-	-	-	-	-	0.89**	0.50**	-0.11	-0.04
LDL	-	-	-	-	-	-	-	0.20	-0.06	0.08
HDL	-	-	-	-	-	-	-	-	-0.33*	-0.44**
VLDL	-	-	-	-	-	-	-	-	-	0.75**
Triglycerides	-	-	-	-	-	-	-	-	-	-

G2: HIV-1^+ individuals without lipodystrophy.

HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density cholesterol.

*: $p<0.05$; **: $p<0.01$.

DISCUSSION

Non-drug-related factors such as gender, age, and HIV-1 infection duration (28) also seem to be associated with these body changes, but in the present study there was no association between gender or age and the presence of lipodystrophy (38). Mean disease duration and mean total antiretroviral therapy time were higher in HIV-1 infected individuals with lipodystrophy (G1) than in those without lipodystrophy (G2). Therefore, there seems to be an association between infection time, antiretroviral therapy time, and lipodystrophy. So, G2 individuals may develop such syndrome sometime in the future. However, mean time since last therapy was not associated with lipodystrophy.

Another important aspect reported by several authors is that lipodystrophy may be related to antiretroviral type (31). In the present study, G1 had fewer patients on AZT and more on d4T, without differences for other drugs used, which agrees with literature, where (24) lipodystrophy is associated with d4T. Joly *et al.* (24) showed differences between AZT and d4T in relation to lipid plasmatic levels, revealing

higher values in patients on d4T. However, analyzing the antiretroviral regimens used, it was not possible to see differences between G1 and G2.

Clinical epidemiological alterations have been related to possible alterations in the lipid and immunological profiles. However, smoking, fever, diarrhea, recent weight loss, nausea, and anorexia did not show differences between groups in the present study, thus eliminating their association with lipodystrophy. On the other hand, sedentarism was high in G1, probably due to advice and orientation that these patients received about practicing exercise. Alcohol consumption can interfere with the analysis of lipid profile metabolic parameters (3), but in the present paper it was absent in G1 and at low proportions in G2. Transmission mechanism in developing countries is generally by heterosexual relationship (40), which was similar to the present study.

Albumin, a classic nutritional reference standard, like a negative acute-phase protein (15), was normal in both groups. Therefore, it probably did not interfere with the overall evaluation of clinical metabolic nutritional and immunological alterations.

Several studies have shown that patients on antiretrovirals have increased serum levels of glucose, triglycerides, total cholesterol, and LDL (21). In the present study, there was no difference in glucose concentrations between groups; mean concentration was within reference values. This agrees with Carr (5), who also reported that hypertriglyceridemia is relatively rare in lipodystrophy patients as most patients can compensate insulin resistance with increased pancreatic insulin secretion.

Mean G2 triglyceride levels were higher than reference values, similar to the results obtained by Pujari *et al.* (37). Total and LDL cholesterol serum levels were within normal values between groups, which agrees with a recent study by Mallon *et al.* (29).

HDL cholesterol serum concentration, when separately analyzed according to gender, was lower in G1; this agrees with Carr *et al.* (7), who reported a tendency to lower serum levels of such variable in HIV-1 infected individuals with lipodystrophy.

Individuals in both groups were eutrophic, which has been reported by several authors (33) who stated there is not necessarily a compromised nutritional state.

Other studies reported an association between CD4 and CD8 T lymphocyte count, viral load and lipodystrophy (27); this was not observed in the present study, in which

patients in both groups had CD4 T lymphocyte count above 380 cells/mm^3 and controlled viremia, thus such variables cannot be associated with lipodystrophy. Lichtenstein *et al.* (27) reported that a fast increase in CD4 T cell count may be a risk factor for lipodystrophy.

Knowledge of the immune profile through cytokine analysis and a possible relationship between lipodystrophy and lipid profile are important but not well-known factors.

Imami *et al.* (22) reported that lipodystrophy associated with HIV could be the result of complex interactions between viral factors and antiretroviral agents. At the beginning of antiretroviral therapy, CD4 T cells are predominantly Th2 profile and primarily secrete anti-inflammatory cytokines such as IL-10, later changing to Th1 profile, secreting inflammatory cytokines such as IL-2, IFN-γ, and TNF-α.

Higher levels of TNF-α and its receptor sTNF R-II were observed in G1, compared to G2. This agrees with other authors, who reported high serum concentrations of TNF-α and sTNF R-II in patients with lipodystrophy under HAART therapy, compared to controls (10), and correlation between peripheral fat loss and high sTNF R-II levels in HIV-1 infected patients with lipodystrophy (32).

Constans *et al.* (13) associated increased TNF-α plasma levels during opportunistic infections with reduced total cholesterol plasma levels in HIV infected patients in the pre-HAART period (13). As a consequence, increases in IFN-α and TNF-α serum levels, which occurred secondarily to HIV-1 infection, seem to be related to an alteration in lipid metabolism. However, Ledru *et al.* (26) demonstrated that apoptosis plays an essential role in the negative regulation of TNF-α synthesis by T cells. During natural progression of HIV infection, the susceptibility to apoptosis of TNF-α-producing cells progressively increased, correlating with lower serum levels (26). Apoptosis in TNF-α-producing T cells is highly suppressed by antiretroviral therapy through the progressive accumulation of such cytokine in the blood, therefore, creating a pro-inflammatory environment that can contribute to lipodystrophy development with immunological restoration. Also, such polarization of T cells to TNF synthesis could favor the development of lipodystrophy syndrome by contributing to lipid metabolism alteration (25). However, *in vitro* studies have suggested that TNF-α is not involved with fat accumulation in the breasts or decrease in the lower limbs, seen as early changes in women on HAART. Lipodystrophy, then, could have a

multifactorial pathogenesis, and increased breast fat in these women could represent a different syndrome (16, 19).

Although their *in vivo* action is unclear, cytokines such as IL-2 play a crucial role in promoting both the survival and proliferation of lymphocytes and cytokine promoters, which activate lymphocytes highly susceptible to apoptosis (4). Higher levels of such cytokine were observed in G2, compared to G1. Therefore, low IL-2 and IFN-γ serum concentrations, as per Ostrowsk *et al.* (34), are associated with the progression of HIV-1 infection, which suggests that the disease was more advanced in G1.

High levels of IL-10, TNF-α and its soluble receptor, and the lower IFN-γ and IL-2 production in lipodystrophy patients suggest that IL-10 participates in the control of inflammatory cytokine synthesis, as it can suppress IFN-γ response (16, 18). Different cell types such as T $CD4^+$, $CD8^+$, and NK secrete IFN-γ, which has a negative regulatory effect on Th2-profile cytokine secretion (8). In the present study, higher IL-10 levels in G1 than in G2 could suggest a regulatory effect over IFN-γ production in G1. Also, TNF-α and IFN-γ can induce virus-infected target cell death by cytotoxic T lymphocyte (23). In another study on IFN-γ participation in lipodystrophy, Clerici *et al.* (12) reported that some important immune functions, including IL-2 and IFN-γ production, remain compromised in AIDS women whether they have lipodystrophy or not. Similar results were found in the present study in the lipodystrophy group (G1).

However, G2 patients had significantly higher levels of IFN-γ and IL-2 associated with lower production of TNF-α and its receptor, suggesting that TNF-α synthesis is controlled in these patients. Lower TNF-α serum concentration in G2 patients seems to show its participation in the onset of such metabolic disorder.

According to some authors (9, 20), most hypertriglyceridemia cases are associated with immune dysfunction. But, in the present study, it was not possible to associate the immunological profile with the lipid profile, as the cytokines analyzed in these individuals did not correlate to the lipid profile. Finally, such data suggest G1 patients had high serum concentrations of TNF-α, its receptor, and IL-10, and low concentrations of IL-2 and IFN-γ, compared to G2.

Therefore, we believe that different TNF-α-producing cell populations need to be studied to help understand the mechanisms leading to lipodystrophy in HAART patients.

REFERENCES

1 ANDRE P., GROETTRUP M., KLENERMAN P., DE GIULI R., BOOTH BL., CERUNDOLO V., BONNEVILLE M., JOTEREAU F., ZINKERNAGEL RM., LOTTEAU V. An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation and T cell responses. *Proc. Natl. Acad. Sci*., 1998, 95, 13120-4.

2 BEHRENS G., DEJAM A., SCHMIDT H. Impaired glucose tolerance beta cell function and lipid metabolism in HIV patients under treatment with protease inhibitors. *AIDS*, 1999, 10, 63-70.

3 BELL RA., MAYER-DAVIS EJ., MARTIN MA., D'AGOSTINO RB., HAFFNER SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the insulin resistance and atherosclerosis study. *Diabetes Care*, 2000, 23, 1630-6.

4 BOISE LH., THOMPSON CB. Hierarchical control of lymphocyte survival. *Science*, 1996, 274, 67-8.

5 CARR A. HIV Protease Inhibitor-Related Lipodystrophy Syndrome. *Clin. Infect. Dis.*, 2000, 30, S135-42.

6 CARR A., MILLER J., LAW M., COOPER DA. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. *AIDS,* 2000, 14, 25-32.

7 CARR A., SAMARAS K., THORISDOTTIR A., KAUFMANN GR., CHISHOLM DJ., COOPER DA. Diagnosis, prediction, and natural course of HIV-1 protease inhibitor-associated lipodystrophy, hyperlipidemia, and d*iabetes mellitus*: a cohort study. *Lancet,* 1999, 353, 2093-9.

8 CHOMARAT P., RISSOAN MC., BANCHEREAU J., MOSSEC P. IFN-[gamma] inhibits IL-10 production by monocytes. *J. Exp. Med.*, 1993, 177, 523-7.

9 CHRISTEFF N., LORTHOLARY O., CASASSUS P. Serum lipid concentration with reference to the clinical and immunological status of HIV infected men. *Ann. Intern. Med.,* 1995, 7, 490-5.

10 CHRISTEFF N., MELCHIOR JC., TRUCHIS P., PERRONNE C., GOUGEON ML. Increased serum interferon-alpha in HIV-1 associated lipodystrophy syndrome. *Eur. J. Clin. Invest.*, 2002, 32, 43-50.

11 CHRISTEFF N., MELCHIOR JC., TRUCHIS P., PERRONNE C., NUNEZ EA., GOUGEON ML. Lipodystrophy defined by a clinical score in HIV-infected men on highly active antiretroviral therapy: correlation between dyslipidaemia and steroid hormone alterations. *AIDS*, 1999, 13, 2251-60.

12 CLERICI M., GALLI M., BOSIS S., GERVASONI C., MORONI M., NORBIATO G. Immunoendocrinologic abnormalities in human immunodeficiency virus infection. *Ann. N. Y. Acad. Sci.*, 2000, 917, 956-61.

13 CONSTANS J., PELLEGRIN JL., PEUCHANT E. Plasma lipids in patients infected with HIV: a prospective study in 95 patients. *Eur. J. Clin. Invest.*, 1994, 6, 416-20.

14 CURI PR. *Metodologia e Análise da Pesquisa em Ciências Biológicas*. 2.ed. Botucatu: Tipomic, 1998. 263p.

15 DOWEIKO JP., NOMPLEGGI DJ. Role of albumin in human physiology and pathophysiology. *J. Parenter. Enteral. Nutr*., 1991, 15, 207-11.

16 FEINGOLD KR., SOUED M., SERIO MK., MOSER AH., DINARELLO CA., GRUNFELD C. Multiple cytokines stimulate hepatic lipid synthesis *in vivo*. *Endocrinology,* 1989, 125, 267-74.

17 FINZI D., BLANKSON J., SILICIANO JD., MARGOLICK JB., CHADWICK K., PIERSON T., SMITH K., LISZIEWICZ J., LORI F., FLEXNER C., QUINN TC., CHAISSON RE., ROSENBERG E., WALKER B., GANGE S., GALLANT J., SILICIANO RF. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat. Med.,* 1999, 5, 512-17.

18 FIORENTINO D., ZLOTNIK F., MOSMANN TR. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.,* 1991, 147, 3815-21.

19 GALLI M., GERVASONI C., RIDOLFO AL., TRABATTONI D., SANTAMBROGIO S., VACCAREZZA M., MERONI L., TRIFIRO G., MORONI M., NORBIATO G., CLERICI M. Cytokine production in women with antiretroviral treatment-associated breast fat accumulation and limb wasting. *AIDS*, 2003, 17, 155-61.

20 GRUNFELD C., PANG M., DOERRLER W., SHIGENAGA JK., JENSEN P., FEINGOLD KR. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.*, 1992, 74, 1045-52.

21 GRUNFELD C., SOUED M., ADI S., MOSER AH., DINARELLO CA., FEINGOLD KR. Evidence for two classes of cytokines that stimulate hepatic lipogenesis: relationships among tumor necrosis factor, interleukin-1 and interferon-alpha. *Endocrinology*, 1990, 127, 46-54.

22 IMAMI N., ANTONOPOULOS C., HARDY GA., GAZZARD B., GOTCH FM. Assessment of type-1 and type-2 cytokines in HIV type-1-infected individuals: impact of highly active antiretroviral therapy. *AIDS Res. Hum. Retroviruses*, 1999, 15, 1499-508.

23 IMAMI N., GOTCH F. Prospects for immune reconstitution in HIV-1 infection. *Clin. Exp. Immunol.*, 2002, 127, 402-11.

24 JOLY V., FLANDRE P., MEIFFREDY V., LETURQUE N., HAREL M., ABOULKER JP., YENI P. Increased risk of lipoatrophy under stavudine in HIV-1-infected patients: results of a substudy from a comparative trial. *AIDS*, 2002, 16, 2447-54.

25 LEDRU E., CHRISTEFF N., PATEY O., DE TRUCHIS P., MELCHIOR JC., GOUGEON ML. Alteration of tumor necrosis factor-α T-cell homeostasis following potent antiretroviral therapy: contribution to the development of human immunodeficiency virus-associated lipodystrophy syndrome. *Blood*, 2000, 95, 3191-8.

26 LEDRU E., LECOEUR H., GARCIA S., DEBORD T., GOUGEON ML. Differential susceptibility to activation-induced apoptosis among peripheral Th1 subsets: correlation with Bcl-2 expression and consequences for AIDS pathogenesis. *J. Immunol*., 1998, 160, 3194-206.

27 LICHTENSTEIN KA., WARD DJ., MOORMAN AC., DELANEY KM., YOUNG B., PALELLA JUNIOR FJ., RHODES PH., WOOD KC., HOLMBERG SD. Clinical assessment of HIV-associated lipodystrophy in an ambulatory population. *AIDS,* 2001, 15, 1389-98.

28 LO JC., MULLIGAN K., TAI VW., ALGREN H., SCHAMBELAN M. “Buffalo hump” in men with HIV-1 infection. *Lancet,* 1998, 351, 867-70.

29 MALLON PWG., WAND H., LAW M., MILLER J., COOPER DA., CARR A. Buffalo hump seen in HIV-associated lipodystrophy is associated with hyperinsulinemia but not dyslipidemia. *J. Acquir. Immune. Defic. Syndr.*, 2005, 38, 156-2.

30 MEHTA S., MOORE RD., GRAHAM NMH. Potential factors affecting adherence with HIV therapy. *AIDS*, 1997, 11, 1665-70.

31 MILLER KD., JONES E., YANOVSKI JA. Visceral abdominal-fat accumulation associated with use of indinavir. *Lancet*, 1998, 351, 871-5.

32 MYNARCIK D., MCNULAN M., STEIGBIGEL R. Association of severe insulin resistance with both loss of limb fat and elevated serum tumor necrosis factor receptor levels in HIV lipodystrophy. *J. Acquir. Immune. Defic. Syndr.*, 2000, 25, 312-21.

33 MYNARCIK D., WEI LX., KOMAROFF E., FERRIS R., MCNURLAN M., GELATO M. Chronic loss of subcutaneous adipose tissue in HIV-associated lipodystrophy may not be associated with accelerated apoptosis. *J. Acquir. Immune. Defic. Syndr.*, 2005, 38, 367-71.

34 OSTROWSKI SR., GERSTOFT J., PEDERSEN BK., ULLUM H. Impaired production of cytokines is an independent predictor of mortality in HIV-1-infected patients. *AIDS*, 2003, 17, 521-30.

35 PALELLA JUNIOR FJ., DELANEY KM., MOORMAN AC., LOVELESS MO., FUHRER J., SATTEN GA., ASCHMAN DJ., HOLMBERG SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV outpatient study investigators. *N. Engl. J. Med.,* 1998, 338, 853-60.

36 PONTES LCR., CARVALHO LR., SOUZA LR., TRINDADE EBSM., PEREIRA PCM. Lipid profile and body composition of HIV-1 infected patients treated with highly active antiretroviral therapy. *J. Venom. Anim. Toxins inc. Trop. Dis*., 2005, 11, 143-59.

37 PUJARI SN., DRAVID A., NAIK E., BHAGAT S., TASH K., NADLER JP., SINNOTT JT. Lipodystrophy and dyslipidemia among patients taking first-line, World Health Organization-recommended highly active antiretroviral therapy regimens in Western India. *J. Acquir. Immune. Defic. Syndr.*, 2005, 39, 199-202.

38 SAINT-MARC T., PARTISANI M., POIZOT-MARTIN I., BRUNO F., ROUVIERE O., LANG JM., GASTAUT JA., TOURAINE JL. A syndrome of peripheral fat wasting

(lipodystrophy) in patients receiving long-term nucleoside analogue therapy. *AIDS,* 1999, 13, 1659-67.

39 SLOAND EM., KUMAR PN., KIM S., CHAUDHURI A., WEICHOLD FF., YOUNG NS. HIV-1 protease inhibitor modulates activation of peripheral blood CD4+ T cells and decreases their susceptibility to apoptosis *in vitro* and *in vivo*. *Blood,* 1999, 94, 1021-7.

40 WORLD HEALTH ORGANIZATION. Joint United Nations Program on HIV/AIDS (UNAIDS). AIDS epidemic update: December 2001. Geneva, [serial on-line], 2005. [cited 2005 Nov. 23]. Available from: http://img.thebody.com/legacyAssets/07/79/update1201.pdf.