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Tumor necrosis factor α is associated with insulin-mediated suppression of free fatty acids and net lipid oxidation in HIV-infected patients with lipodystrophy

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Abstract

Tumor necrosis factor α (TNF-α) stimulates lipolysis in man. We examined whether plasma TNF-α is associated with the degree by which insulin suppresses markers of lipolysis, for example, plasma free fatty acid (FFA) and net lipid oxidation (LIPOX) rate in HIV-infected patients with lipodystrophy (LIPO) and those without (controls). LIPOX was estimated by indirect calorimetry during fasting and steady state of a hyperinsulinemic euglycemic clamp in 36 (18 LIPO and 18 controls) normoglycemic HIV-infected men on highly active antiretroviral therapy. In LIPO, TNF-α correlated with clamp FFA ($r = 0.67$, $P < .01$), clamp LIPOX ($r = 0.47$, $P < .05$), incremental FFA ($r = 0.69$, $P < .01$), and incremental LIPOX ($r = 0.64$, $P < .01$), all of which, but not the clamp LIPOX correlation ($r = 0.29$, $P > .05$), remained significant after correction for insulin sensitivity. None of these correlations were significant in controls. In all patients, TNF-α correlated with clamp FFA ($r = 0.61$, $P < .001$), clamp LIPOX ($r = 0.43$, $P < .01$), and incremental FFA ($r = 0.43$, $P < .01$), with the 2 former correlations remaining significant after correction for insulin sensitivity. LIPOX and FFA (fasting and clamp values combined) correlated strongly and positively in both LIPO ($R^2 = 0.43$, $P < .001$) and controls ($R^2 = 0.60$, $P < .0001$). Fasting FFA and LIPOX did not differ between study groups; however, the insulin-mediated suppression of FFA and LIPOX was attenuated in LIPO (P's $< .05$). Our data indicate that higher TNF-α, independently of insulin sensitivity, is associated with attenuated insulin-mediated suppression of FFA and LIPOX in HIV-LIPO, suggesting in turn that TNF-α stimulates lipolysis in this syndrome. Furthermore, FFA may be a major determinant of LIPOX in HIV-infected patients on highly active antiretroviral therapy.
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1. Introduction

Lipodystrophy (LIPO), most frequently in the combined form of peripheral fat atrophy and central fat accumulation [1], affects more than 40% of HIV-infected patients who has been on highly active antiretroviral therapy (HAART) for 1 year or more [2]. Lipolysis, which is often increased in HIV-infected patients on HAART [3-6], may play an important role for the lipodystrophic phenotype of HIV-infected patients in terms of accelerating fat redistribution [3,5,7]. Tumor necrosis factor α (TNF-α) enhances lipolysis from adipose tissue in man [8-10]. High plasma TNF-α levels have been reported in HIV-LIPO compared with nonlipodystrophic HIV-infected patients and healthy control subjects [11,12]. We have previously shown a positive

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correlation between expression of TNF-α messenger RNA (mRNA) in subcutaneous adipose tissue and plasma TNF-α in HIV-infected patients on HAART [13], suggesting that increased plasma TNF-α may relate to an increased release of TNF-α from adipose tissue.

The present study aimed to examine the association between plasma TNF-α level and markers of lipolysis, that is, net LIPOX, plasma free fatty acids (FFAs), and glycerol in the postabsorptive phase and after insulin-mediated suppression of lipolysis in a group of HIV-infected patients on HAART, who either did or did not display LIPO. Second, it was examined whether net LIPOX correlates with plasma FFA in HIV-infected patients on HAART, as has been shown in HIV-negative individuals [14]. Third, it was addressed whether plasma TNF-α correlates with plasma triglyceride in HIV-infected patients on HAART, as TNF-α has been demonstrated to be involved in hypertriglyceridemia [15]. Finally, it was examined whether plasma triglyceride correlated inversely with high-density lipoprotein cholesterol (HDL-C), which indirectly would support a role for triglyceride in lowering HDL-C in such patients in accordance with previous findings in HIV-negative individuals [16].

2. Methods

2.1. Subjects

Eighteen normoglycemic HIV-1–infected male patients on HAART (LIPO), who displayed fat redistribution, were recruited from the outpatient clinic of infectious diseases, Hvidovre Hospital, University of Copenhagen, Hvidovre, Copenhagen, Denmark. Likewise, 18 normoglycemic HIV-1–infected male patients without signs of LIPO were recruited as control subjects. All participants were on HAART. A description of selection, anthropometry, immunology, and components of HAART of all subjects has been presented [17]. In brief, for cases, a questionnaire and a physical examination had to be positive for signs of LIPO, whereas for control subjects, both the questionnaire and physical examination had to be negative. All cases, except one who had multiple lipomatosis, displayed LIPO consistent with peripheral fat atrophy and central fat accumulation (ie, mixed LIPO [1]). Except for HAART, none of the subjects received medication known to affect glucose metabolism. All participants, except for 2 control subjects, had a negative family history of diabetes mellitus, and all participants except for 2 control subjects (Black Africans) were of white ethnicity. Subjects gave their written informed consent, and the protocol was approved by the Ethical Committee in Copenhagen, Denmark, and performed in accordance with the Helsinki Declaration II.

2.2. Experimental protocol

All subjects abstained from strenuous exercise for at least 3 days before the metabolic assessments. The HIV-infected patients reported to the laboratory at 8:00 AM after a 12-hour overnight fast, including a 16-hour withdrawal of HAART. At 8:30 AM, 2 intravenous cannulas were inserted, one in a dorsal hand vein and the other in the contralateral antecubital vein. The hand vein was used for blood sampling, whereas the antecubital vein was used for infusion. At 9:00 AM, a 150-minute basal period (0-150 minutes) started, which was followed by an intravenous glucose tolerance test (0.3 g/kg body weight, 150-180 minutes) and a 120-minute hyperinsulinemic euglycemic clamp (180-300 minutes). Two steady-state periods were predefined, that is, at 90 to 120 minutes (fasting) and at 270 to 300 minutes (clamp). During both steady-state periods, indirect calorimetry was performed using a computerized canopy gas analyzer system (Deltatrac II Metabolic Monitor; Datex, Helsinki, Finland) to estimate net lipid oxidation (LIPOX) rate as outlined by Frayn [18]. Insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) infusion was started at 100 mU $\cdot$ m^{-2} $\cdot$ min^{-1} and reduced stepwise to 40 mU $\cdot$ m^{-2} $\cdot$ min^{-1} during the first 9 minutes of the clamp period; hereafter (9-120 minutes), the insulin infusion rate was fixed at 40 mU $\cdot$ m^{-2} $\cdot$ min^{-1}. Plasma glucose concentration was kept at approximately 5 mmol/L by adjusting the infusion rate of glucose (180 g/L). During the clamp period, glucose levels were determined every 5 minutes, and blood samples for assessing plasma insulin levels were taken every 10 minutes. Blood samples for assessing plasma FFAs, glycerol, triglyceride, and HDL-C were taken during the basal fasting period at 90 and 120 minutes, and during the clamp period at 270 and 300 minutes. At baseline (ie, between 120 and 150 minutes), adipose tissue biopsies were taken from the subcutaneous abdominal region (periumbilically). After application of local anesthesia (5 mg/mL lidocaine), the biopsies were taken by needle aspiration (liposuction). The adipose tissue was washed thoroughly with isotonic saline and then frozen in liquid nitrogen and kept at 80°C for later RNA extraction.

Body composition was estimated by dual energy x-ray absorptiometry scanning (XR-36; Norland Medical System, Fort Atkinson, WI). A whole-body scan was performed to estimate the amount of fat in the trunk, abdomen, and extremities. The proximal limitation of the leg region was placed through the hip joints at an angle of approximately 45°. To assess the distribution of visceral abdominal adipose tissue and subcutaneous abdominal adipose tissue, a single-slice computed tomography scan at the L4 vertebral level was performed in 16 LIPO patients and in 15 control subjects. The area of adipose tissue was measured in square centimeters.

2.3. Assays

Whole blood glucose levels were determined pairwise on 2 calibrated HemoCue B-Glucose Analyzers (HemaCue, Angelholm, Sweden) with an intra-analyzer coeffi-

cient of variation (CV) of 3.5% and an interanalyzer CV of 3.3%. Plasma glucose was calculated using previously validated equations [19]. Blood samples were centrifuged immediately at 4°C, and plasma was stored at −80°C for later analysis. Plasma insulin concentrations were determined by the 1235 AutoDELPHIA automatic immunoassay system (Wallac Oy, Turku, Finland). The insulin assay had a detection limit of approximately 3 pmol/L. Cross-reactivity with intact proinsulin was 0.1%, 0.4% with 32 to 33 split proinsulin, and 66% with 64 to 65 split proinsulin; the intra-assay CV was 4.5% and the inter-assay CV was 7%. Plasma FFAs were determined using an enzymatic colorimetric method (Wako C test kit, Wako Chemicals, Neuss, Germany) with an inter-assay CV of 5%. Plasma HDL-C and triglyceride were determined by reflection photometry (Ortho-Clinical Diagnostics kit, Raritan, NJ) with inter-assay CVs of 8% and 2.5%, respectively. Plasma glycerol was measured by the fluorometric method. CD4 count determination (flow cytometry, Becton-Dickinson FACscan, BD, Franklin Lakes, NJ; inter-assay CV of 7%) and viral load determination (Roche Amplicor Ultrasensitive Assay with a detection limit of 20 copies per milliliter of plasma; Roche, Basel, Switzerland) met the requirements of interlaboratory quality control. TNF-α plasma concentrations were measured using human enzyme-linked immunosorbent assays (Quantikine HS ELISA, R&D Systems Europe, Abingdon, UK). Adipose tissue mRNA expression of TNF-α was determined using real-time polymerase chain reaction as described [13].

2.4. Calculations

The parameter denoted as limb fat (%), which was calculated as the extremity fat divided by the sum of trunk fat and extremity fat, was taken as an indicator of severity of LIPO. *M* value was calculated as the glucose infusion rate during clamp steady state normalized for fat free mass. "Incremental" or "Δ" data signify the clamp value minus the baseline (fasting) value.

2.5. Statistical analysis

Data are presented as means ± SEM, if not otherwise indicated. Analysis of variance was performed to compare distribution of data between the LIPO group and control subjects. As it appeared that age was increased in LIPO compared with control subjects, we adjusted for age by univariate analysis of variance (in Table 1), with patient group (qualitative factor) and age (quantitative factor) as covariates. Pearson correlation coefficient (*r*) was calculated for measurements of associations between variables. Adjustments for insulin sensitivity and incremental insulin were performed, calculating the partial correlation coefficients between variables. If data distribution was skewed, data are given as medians and 25th and 75th percentiles, and comparison of data distribution between study groups was estimated by Mann-Whitney *U* test. Calculations were performed by SPSS (SPSS ver 12.0; SPSS, Chicago, IL). Two-sided *P* values of less than .05 were defined as statistically

Table 1
Anthropometry, lipidemia, and immunology of study groups

	LIPO	Controls	*P*
n	18	18	
Age (y)	50 (2)	43 (2)	<.05
BMI (kg/m^2)	24.7 (0.6)	22.6 (0.8)	<.05
Total fat mass (kg)	16 (1)	13 (2)	.13
Total lean mass (kg)	61 (2)	54 (2)	<.05*
Limb fat (%)	36 (1)	46 (2)	<.0001***
$CT_{visceral\ fat}$ (cm^2)	203 (26)[a]	68 (15)[b]	<.001***
$CT_{subcutaneous\ fat}$ (cm^2)	102 (15)[a]	102 (22)[b]	NS
$CT_{visceral\ fat}$/BMI	8.1 (0.9)[a]	2.8 (0.5)[b]	<.0001***
AT–TNF-α mRNA (log 10 AU)	1.40 (0.07)	1.15 (0.07)	<.05*
fP–TNF-α (log 10 pg/mL)	0.42 (0.06)	0.32 (0.07)	NS
fP-TG (log 10 mmol/L)	0.47 (0.08)	0.30 (0.06)	.09
cP-TG (log 10 mmol/L)	0.44 (0.08)	0.24 (0.07)	.08
fP–HDL-C (mmol/L)	0.97 (0.10)	1.01 (0.07)	NS
fP-FFA (log 10 μmol/L)	2.72 (0.04)	2.71 (0.04)	NS
cP-FFA (log 10 μmol/L)	2.11 (0.06)	1.91 (0.05)	<.05*
ΔFFA (%)	−73 (3)	−81 (3)	<.05*
fP-glycerol (log 10 μmol/L)	1.81 (0.04)	1.82 (0.03)	NS
cP-glycerol (log 10 μmol/L)	1.60 (0.06)	1.48 (0.05)	.16
Δglycerol (%)	−31 (−56, −26)	−60 (−69, −30)	<.05
Fasting LIPOX (mg · min^{-1} · m^{-2})	28 (2)	28 (3)	NS
Clamp LIPOX (mg · min^{-1} · m^{-2})	17 (2)	11 (3)	.08
ΔLIPOX (mg · min^{-1} · m^{-2})	−11 (2)	−18 (2)	<.05*
fP-insulin (mmol/L)	77 (11)	32 (4)	<.001**
cP-insulin (mmol/L)	535 (35)	392 (16)	<.001**
ΔP-insulin (mmol/L)	458 (27)	360 (15)	<.01*
fP-glucose (mmol/L)	4.9 (0.1)	4.8 (0.1)	NS
M value (mg · min^{-1} · kg_{FFM}^{-1})	5.5 (0.5)	8.2 (0.5)	<.001**
CD4 (cells/μL)	427 (45)	352 (46)	NS
HIV RNA (copies/mL)	<20 (<20, 70)	<20 (<20, <20)	NS
Duration of HIV infection (mo)	99 (14)	72 (11)	.16
Duration of NRTI therapy (mo)	47 (7)	42 (6)	NS
Duration of PI therapy (mo)	32 (4)	25 (4)	.16

Data are presented as mean (SEM) or median (25th and 75th percentile). Asterisks after the *P* values indicate significance levels after adjustment for age. CT indicates computed tomography; AT, adipose tissue; AU, arbitrary unit; fP, fasting plasma; cP, clamp plasma; TG, triglyceride; Δ, clamp value minus fasting value; *M* value, insulin sensitivity estimated by euglycemic clamp technique.

[a] n = 16.

[b] n = 15.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

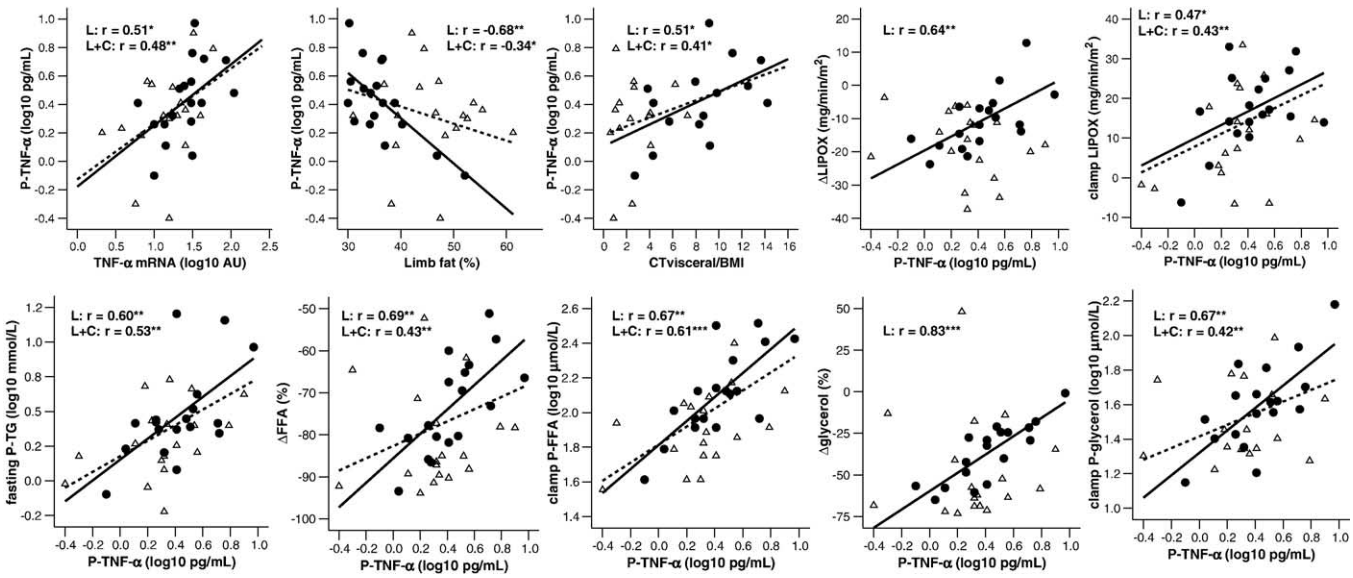

Fig. 1. Scatterplots showing correlations between plasma TNF-α vs expression of TNF-α in adipose tissue, measures of fat distribution, and markers of lipolysis in the LIPO group (●) and the control subjects (△). Pearson correlation coefficients are given in each panel. Solid lines indicate significant correlations for the LIPO group (L); dashed lines, for the combined group of LIPO and control subjects (L + C); P–TNF-α, plasma TNF-α. Other abbreviations are given in Table 1. *$P < .05$; **$P < .01$; ***$P < .001$.

significant. A trend was noted for P values of .05 or greater, but less than .2.

3. Results

Table 1 provides comparative data of the study groups showing that patients with LIPO were slightly older than controls and had higher body mass index (BMI), of which the latter could be explained by an increased lean body mass in the LIPO group. Limb fat was decreased in the LIPO group, and visceral fat mass increased 3-fold, whereas total fat mass did not differ significantly between study groups. Expression of TNF-α in adipose tissue and plasma TNF-α were increased in the LIPO group, although the latter did not reach statistical significance. A description of metabolic data, data from the intravenous glucose tolerance test, fat distribution, and the components of HAART of the HIV-infected patients has been presented [17,20,21]. HIV RNA and CD4 including duration of HIV infection, HIV-1 protease inhibitor (PI), and nucleotide reverse transcriptase inhibitor (NRTI) therapy did not differ significantly between study groups. All patients were on NRTI therapy. Nucleotide reverse transcriptase inhibitors used frequently in the LIPO and non-LIPO groups were those of lamivudine (83%, 83%), zidovudine (39%, 50%), and stavudine (56%, 39%), respectively. As part of HAART, PIs were used by 89% in the LIPO and non-LIPO groups, that is, indinavir (44% and 22%), ritonavir (22% and 39%), nelfinavir (22% and 22%), and saquinavir (17% and 11%), respectively.

The insulin-mediated suppression of LIPOX, plasma FFA, and glycerol was attenuated in the LIPO group compared with control subjects despite a greater increment in insulin in the LIPO group (Table 1). Plasma FFA during the clamp period was significantly increased in the LIPO group, and glycerol and LIPOX tended to be

Table 2
Pearson correlation coefficients among TNF-α, limb fat, visceral abdominal fat, and insulin sensitivity vs measures of lipid metabolism in LIPO

			ΔLIPOX	Clamp LIPOX	fP-TG	ΔFFA (%)	cP-FFA	Δglycerol (%)	cP-glycerol
0.51*	0.68**	P–TNF-α (unadjusted/adjusted for M value)	0.64**/ 0.54*	0.47*/ 0.29	0.60**/ 0.56*	0.69**/ 0.59*	0.67**/ 0.65**	0.83***/ 0.77***	0.67**/ 0.59*
	0.52*	Limb fat (%)	−0.50*	−0.54*	−0.44	−0.39	−0.42	−0.64**	−0.48*
		$CT_{visceral\ fat}$/BMI[a]	0.29	0.47	0.10	0.51*	0.44	0.39	0.34
M value			−0.42	−0.52**	−0.26	−0.47*	−0.65**	−0.56*	−0.52*

Number of patients is 18 if not otherwise noted. The correlations for plasma TNF-α are indicated both as unadjusted and as adjustment for insulin sensitivity (*M* value).

[a] n = 16.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

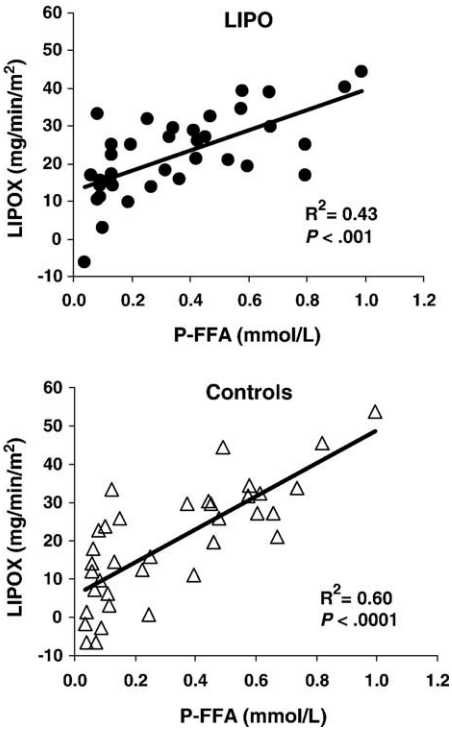

Fig. 2. Correlations between fasting and clamp LIPOX vs fasting and clamp plasma FFA in the LIPO group (●; upper scatterplot) and the control subjects (△; lower scatterplot). Correlation coefficients are given as R^2.

increased in the LIPO group compared to controls. The LIPO group displayed more than 30% lower insulin-mediated glucose disposal rate than control subjects, despite fasting normoglycemia in both study groups. Of notice, steady state of plasma glucose and insulin was established in both study groups during the latter 75 minutes of the clamp period.

Fig. 1 shows scatterplots indicating that plasma TNF-α correlated positively with adipose tissue TNF-α expression and area of visceral abdominal fat normalized for BMI in the LIPO group (n = 18) and the combined group (n = 36), whereas the correlation between plasma TNF-α and limb fat was negative in the LIPO group and the combined group. Furthermore, Fig. 1 depicts the correlations of plasma TNF-α vs markers of lipolysis, including incremental and clamp LIPOX, FFA, glycerol, and fasting triglyceride, all of which were positive and significant in the LIPO group. In the combined group, most of these correlations appeared significant. By contrast, in control subjects, these correlations did not reach statistical significance.

Table 2 shows the correlations of plasma TNF-α, limb fat, visceral adipose tissue, and *M* value vs markers of lipolysis for the LIPO group only. Correlations between plasma TNF-α vs markers of lipolysis (see also Fig. 1), in general, were stronger than correlations between measures of fat distribution and *M* value vs markers of lipolysis. *M* value and plasma TNF-α correlated significantly and inversely in the LIPO group ($r = -0.50$, $P < .05$). However, correcting for insulin sensitivity (*M* value), all correlations between plasma TNF-α and markers of lipolysis remained significant except for clamp LIPOX. Moreover, the correlations between plasma TNF-α and markers of lipolysis remained significant in the LIPO group after correction for both incremental and clamp plasma insulin levels (data not shown).

The correlations between adipose tissue TNF-α expression vs markers of lipolysis in the LIPO group were significant for clamp FFA ($r = 0.56$, $P < .05$), clamp glycerol ($r = 0.52$, $P < .05$), and Δglycerol ($r = 0.47$, $P < .05$); however, these correlations were not significant after correcting for *M* value. The correlation between adipose tissue TNF-α expression vs *M* value was borderline significant in LIPO ($r = -0.45$, $P = .061$).

In the combined group (n = 36), the *M* value tended to correlate significantly with plasma TNF-α ($r = -0.30$, $P = .08$). After correction for *M* value, the following correlations between plasma TNF-α and markers of lipolysis appeared significant in the combined group: plasma TNF-α vs clamp LIPOX ($r = 0.35$, $P < .05$); plasma TNF-α vs fasting triglyceride ($r = 0.48$, $P < .005$); and plasma TNF-α vs clamp FFA ($r = 0.58$, $P < .001$). All these correlations remained significant after correction for incremental and clamp insulin concentrations.

In the combined group, *M* value correlated significantly with adipose tissue expression of TNF-α ($r = -0.35$, $P < .05$). Among the markers of lipolysis, adipose tissue TNF-α

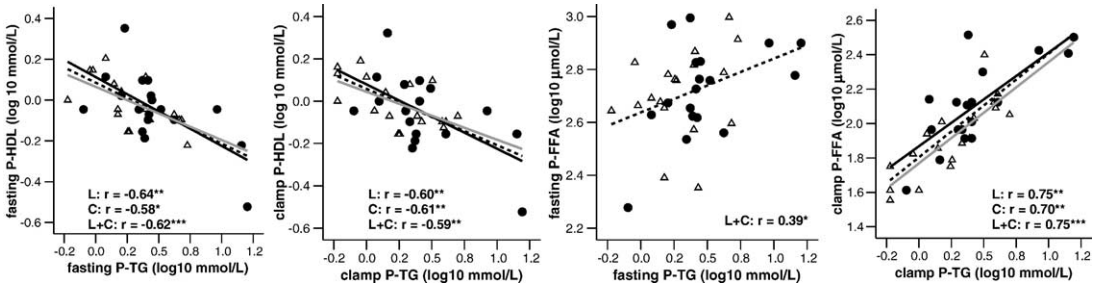

Fig. 3. Scatterplots showing correlations between plasma triglyceride and plasma HDL-C and FFA during fasting and clamp periods in the LIPO group (●) and the control subjects (△). Pearson correlation coefficients are given in each panel. Solid black lines indicate significant correlations for the LIPO group (L); solid gray lines, for control subjects (C); dashed lines, for the combined group of LIPO and control subjects (L + C). *$P < .05$; **$P < .01$; ***$P < .001$.

expression correlated significantly and positively with clamp LIPOX and clamp FFA in the combined group. Both these correlations were robust for correction for incremental and clamp insulin levels. However, after correction for *M* value, the positive correlation between adipose tissue TNF-α expression vs clamp LIPOX was the only correlation that remained significant in the combined group (data not shown).

Fig. 2 presents the correlation between fasting and clamp LIPOX vs fasting and clamp plasma FFA in the LIPO group (upper graph) and the control subjects (lower graph), showing that the variation of plasma FFA may explain a major fraction of the variation of LIPOX, that is, 43% in the LIPO group and 60% in control subjects.

Fig. 3 shows a strong correlative relationship between plasma triglyceride and HDL-C by using scatterplots, both during fasting and clamp periods in the LIPO group, control subjects, and the combined group. Fasting FFA correlated significantly with fasting plasma triglyceride in the combined group, and during clamp period, the correlations between these metabolites were strong both in the LIPO group and the control subjects, and when groups were combined.

4. Discussion

The present cross-sectional study provides detailed analysis of the relationship between plasma TNF-α and markers of lipolysis in HIV-infected patients with and without LIPO. Most importantly, plasma TNF-α, independent of incremental insulin concentration and insulin sensitivity, correlated strongly and negatively with the degree by which insulin reduced FFA, glycerol, and net LIPOX in HIV-infected patients with LIPO, suggesting that plasma TNF-α attenuates the ability of insulin to suppress lipolysis in such patients.

TNF-α is associated with the rate of lipolysis from adipose tissue by activating mitogen protein kinases [10]. An increased intra-abdominal fat mass in man is associated with higher plasma TNF-α concentrations [22-24] and increased release of FFA [25]. Our data may be of clinical importance because they suggest that the severity in fat redistribution toward a lipodystrophic phenotype with increased intra-abdominal fat mass and low relative fat mass in the extremities is related to increased expression of TNF-α in adipose tissue and plasma TNF-α concentration, in turn showing strong positive correlations to indices of lipolysis in patients with HIV-LIPO, particularly during insulin stimulation. Thus, our data support the notion of a link between rate of lipolysis and severity of LIPO, which in part could be mediated by an increased plasma concentration of TNF-α. In line with the present data, we recently reported a reduced ability of insulin to suppress plasma FFA level during an oral glucose tolerance test in lipodystrophic HIV-infected patients compared with their nonlipodystrophic counterparts [26].

In the present study, plasma FFA predicted approximately 40% to 60% of the variation in net LIPOX in HIV-infected patients on HAART both during fasting and insulin stimulation, which is a significantly higher percentage than the 10% reported by Groop et al [14] from a study in healthy individuals. Such strong correlation between plasma FFA and net LIPOX in HIV-infected patients on HAART may suggest that net LIPOX is more dependent on oxidation of plasma FFA in these patients compared with healthy individuals, which in turn would be consistent with an increased rate of lipolysis in normoglycemic HIV-infected patients on HAART compared with healthy individuals [3]. An estimation of the rate and suppression of lipolysis during exogenous insulin stimulation in relation to fat distribution remains to be established in HIV-infected patients on HAART; however, after an oral glucose challenge, lipolysis was increased in lipodystrophic HIV-infected patients compared with healthy control subjects [27].

The class of HIV-1 PIs has been suggested to stimulate lipolysis [6,28-30]. Ninety percent of our HIV-infected patients were treated with PIs. PI-induced lipolysis and LIPO might be associated with greater plasma TNF-α [11,31]. The other main drug class of the HAART of HIV-infected patients, the NRTIs, which was used by all our patients, has also been associated with increased lipolysis [32]. In addition, NRTIs have been shown to induce TNF- α release from adipocytes [33] and peripheral leukocytes in vitro [34], and NRTIs have been associated with LIPO in epidemiological studies [35]. In accordance with the cited data and our present results, we speculate that a low-grade proinflammatory state, possibly in part mediated by the components of HAART, may be a common denominator behind LIPO and rate of lipolysis in HIV-infected patients.

The present study could not demonstrate a correlation between TNF-α and markers of lipolysis in the fasting state. In fact, the LIPO group and the control subjects did not differ in fasting FFA, glycerol, and net LIPOX, which opposes data from a recent study [5], in which a 6-fold greater fasting plasma triglyceride concentration and a 50% greater fasting net LIPOX were observed in lipodystrophic HIV-infected patients compared with healthy individuals. However, fasting and clamp triglyceride concentration merely tended to be 50% greater in the LIPO group compared with control subjects in the present study. TNF-α has been shown to stimulate triglyceride production in vivo [15,36]. Importantly, plasma TNF-α correlated strongly and positively with plasma triglyceride in the combined group and in the LIPO group, suggesting a role for TNF-α in promoting hypertriglyceridemia in such patients. Furthermore, in the combined group of HIV-infected patients on HAART, plasma triglyceride, which was increased by approximately 150% compared with the median of the normal reference range for our laboratory, correlated strongly and negatively with HDL-C, which in turn was

decreased by approximately 25% compared with the median of the normal reference range for our laboratory. An increased level of plasma triglycerides drives the exchange of core lipids between triglyceride-rich lipoprotein and HDL particles, resulting in triglyceride enrichment of HDL particles [37]. As triglyceride enrichment of HDL has been shown to enhance clearance of HDL [38], such mechanism could explain the inverse correlation between plasma triglyceride and HDL-C and the relatively low level of HDL of HIV-infected patients in the present study.

In conclusion, these data suggest that plasma TNF-α concentration is strongly, negatively, and independently associated with the ability of insulin to suppress glycerol, FFAs, and net LIPOX in HIV-LIPO, which support evidence of an association between low-grade proinflammatory response and lipolysis in this phenotype of HIV-infected patients.

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