Elevated Concentrations of Free Fatty Acids Are Associated With Increased Insulin Response to Standard Glucose Challenge in Human Immunodeficiency Virus-Infected Subjects With Fat Redistribution

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Fat redistribution, defined by both increased abdominal visceral fat and/or decreased abdominal, extremity, and facial subcutaneous fat, is increasingly recognized among human immunodeficiency virus (HIV)-infected patients treated with combination antiretroviral therapy. Fat redistribution in this population is associated with insulin resistance and dyslipidemia and is often referred to as the HIV lipodystrophy syndrome (LIPO). Fatty acids are known to modulate insulin resistance in other disease states, but a comprehensive evaluation of fatty acids has not been undertaken among HIV-infected patients with fat redistribution. In this study, we investigated fatty acid concentrations in 64 HIV-infected individuals (45 men and 19 women) with evidence of fat redistribution (LIPO) in comparison to 30 HIV-infected individuals (20 men and 10 women) without evidence of fat redistribution (NONLIPO) and 32 HIV-negative healthy control subjects (C) (21 males and 11 females) of similar age and body mass index (BMI). Glucose, insulin, and free fatty acid (FFA) levels were measured in response to a 75-g oral glucose tolerance test (OGTT) in the LIPO, NONLIPO, and C subjects. In addition, fasting lipids were obtained, and body composition was determined by anthropometric measurements and dual-energy x-ray absorptiometry (DXA). Fasting FFA concentrations were significantly increased in the LIPO group as compared with NONLIPO and C subjects (0.74 ± 0.03 *v* 0.60 ± 0.04 [mean ± SEM] mmol/L, *P* = .002, LIPO *v* NONLIPO; 0.74 ± 0.03 *v* 0.59 ± 0.03 mmol/L, *P* = .001, LIPO *v* C). In contrast, fasting FFA concentrations were not increased in the NONLIPO group (0.60 ± 0.04 *v* 0.59 ± 0.03, *P* = .909, NONLIPO *v* C). Similarly, fasting triglycerides and 120-minute OGTT FFA were significantly increased in the LIPO group as compared with the NONLIPO and C group. FFA decreased in HIV-infected LIPO, NONLIPO, and C subjects in response to OGTT, but the 120-minute FFA concentrations remained significantly elevated in LIPO patients compared with NONLIPO and C subjects. In a multivariate regression model of LIPO patients, fasting FFA (*P* = .027) was a strong independent predictor of insulin area under the curve (AUC), controlling for age, BMI, gender, and body composition (r^2 for model = .31). No differences were observed in FFA concentrations in the LIPO group in an analysis based on current protease inhibitor (PI) use. These data suggest that FFA concentrations are increased in HIV-infected patients with fat redistribution. Increased fasting concentrations of fatty acids are associated with abnormal insulin responses to standard glucose challenge in HIV-infected patients with fat redistribution. Further studies are necessary to determine the mechanism of increased fatty acid concentrations and the role played by increased FFA in mediating insulin resistance in this population.

FAT REDISTRIBUTION IS increasingly recognized among human immunodeficiency virus (HIV)-infected patients treated with combination antiretroviral therapy and is characterized by increased trunk fat, loss of extremity fat, dyslipidemia, and insulin resistance.[1-3] Detailed body composition analysis using computed tomography (CT) and/or magnetic resonance imaging (MRI) imaging suggest that abdominal visceral fat is increased, whereas abdominal subcutaneous and extremity fat are decreased.[4] The specific mechanisms of fat redistribution in this population remain unclear, but may relate to both drug and non-drug factors.[5-7] Furthermore, it is unknown if the observed increase in visceral fat and reduction in subcutaneous fat represent distinct processes or linked phenomenon of a single syndrome. Insulin resistance is significant among patients with HIV lipodystrophy,[1,2,6,8] but the specific mechanisms of insulin resistance in this population are also not known. Increased free fatty acid (FFA) concentrations may contribute to insulin resistance in the HIV lipodystrophy syndrome (LIPO) through effects on hepatic gluconeogenesis, muscle glucose oxidation, or glucose transport.[9-15]

Few studies have investigated FFA concentrations in the HIV lipodystrophy syndrome in relationship to body composition and indices of insulin resistance.[3,16,17] We compared plasma FFA concentrations in a well-characterized cohort of HIV-infected male and female subjects with fat redistribution to both HIV-infected subjects without fat redistribution and HIV-negative healthy controls of similar age and body mass index (BMI) and assessed the relationship of FFA concentrations to indices of insulin resistance.

MATERIALS AND METHODS

HIV-Infected Subjects With Fat Redistribution

Sixty-four patients (45 men and 19 women) with HIV infection reporting recent change in body fat distribution were prospectively evaluated between December 1998 and July 1999 at the Clinical Research Center of the Massachusetts Institute of Technology. Subjects were recruited using community-based advertisements seeking HIV-infected patients with fat redistribution or were referred by their physicians for evaluation of fat redistribution. Subjects were screened by telephone and asked if they had experienced any of the following: (1) loss of fat in the face, (2) increased fat under the chin or back of the neck, (3) increased abdominal girth, (4) increased chest or breast fat, or

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(5) loss of fat in the arms or legs. Subjects who identified a change in fat distribution in any 1 or more body areas were invited to participate, and fat redistribution was confirmed by physical examination in all subjects (see case examination protocol below). Prior to the determination of anthropometric measurements, degree of fat deposition in the trunk and neck or fat atrophy in the extremities and face was objectively rated by a single investigator (C.H.) based on a 0 to 2-point scale with 0.5 point increments. A score of 0 indicated that no discernible change in fat was present, and a score of 2 signified the presence of severe fat deposition and/or atrophy. A composite score was determined by adding up the score at each of the 4 sites (trunk, neck, face, and extremities). Patients with an abnormal umbilical waist-to-hip ratio (for men, greater than 0.9; for women, greater than 0.8) and physical evidence of significant fat deposition were determined to have lipodystrophy. HIV-infected patients without lipodystrophy did not exhibit significant fat redistribution at any site. Mean lipodystrophy scores were significantly different between the HIV-infected groups (see below).

Subjects were excluded if they had changed antiviral medications within 6 weeks of the study, had a history of diabetes mellitus or previous treatment with an antidiabetic agent, had a BMI greater than 35 kg/m^2, reported use of testosterone, estrogen, growth hormone, or other steroids in the past 6 months, had active alcohol or substance abuse, or were not between 18 to 60 years of age. A subsample of the subjects who participated in this evaluation was subsequently enrolled in a treatment study of metformin for HIV lipodystrophy.[18] Furthermore, metabolic and anthropometric data have been published on these subjects, in comparison to age- and BMI-matched subjects of the Framingham Offspring Study.[19] Fatty acid data have not previously been published on this cohort. Written informed consent was obtained from each subject prior to testing in accordance with the Committee on Human Experimentation with Subjects of the Massachusetts Institute of Technology and the Subcommittee on Human Studies at the Massachusetts General Hospital.

HIV-Infected Subjects Without Fat Redistribution

Twenty HIV-infected male subjects and 10 HIV-infected female subjects without any evidence of fat redistribution, but of similar age and BMI as HIV-infected subjects with fat redistribution, served as control groups.[20] With the exception of fat redistribution, these HIV-infected control subjects met the same criteria for eligibility as the HIV-infected case patients.

HIV-Negative Healthy Control Subjects

Thirty-two (21 men and 11 women) HIV-negative healthy subjects of similar age and BMI as HIV-infected subjects with fat redistribution served as additional control subjects. Control subjects were healthy, without major medical illnesses, and were not receiving any medications known to affect glucose or fatty acid metabolism.

Study Procedures

Subjects underwent a complete medical history and physical examination to confirm the presence or absence of fat redistribution. Weight, height, BMI, waist (at the umbilicus), hip, midarm, and midthigh circumference were determined.[19] All measurements were obtained by a licensed nutritionist with the patient undressed, in triplicate, using a tape measure and then averaged. The waist-to-hip ratio was calculated from the waist circumference measured at the umbilicus divided by the hip circumference measured at the horizontal level of maximum extension of the buttocks.[21] Interrater measurements varied by less than 0.5 cm. Following a 12-hour fast, a standard 75-g oral glucose tolerance test (OGTT) was completed according to World Health Organization (WHO) standards[22] with determination of blood glucose and insulin level at 0, 30, 60, 90, and 120 minutes. Fatty acid concentrations were also obtained at 0 and 120 minutes. In addition, fasting triglyceride concentrations were obtained. Dual-energy x-ray absorptiometry (DXA) was performed to determine total and regional fat mass using a Hologic-4500 densitometer (Hologic, Waltham, MA). Regions of interest (including arms, legs, and trunk) were standardized (1995 Users Guide, Hologic). Trunk fat and extremity fat (sum of fat in the arms and legs) measured in grams are reported.

Biochemical and Immunologic Assays

Levels of glucose and triglyceride (Hitachi 917 Autoanalyzer, Boehringer Mannheim, Mannheim, Germany) were determined by methods previously reported.[23] Serum FFA concentrations were drawn into plain serum tubes, centrifuged, and stored at -80°C. Serum FFA concentrations were later measured using an in vitro enzymatic colorimetric assay kit (Wako Chemicals, Richmond, VA). The intra-assay coefficient of variation for FFA ranged from 1.1% to 2.7%. The published normal range for FFA is 0.1 to 0.6 mmol/L. Insulin levels were measured in serum by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Insulin intra-assay and interassay coefficients of variation ranged from 5.0% to 10.0%, and cross reactivity with proinsulin at midcurve was at least 40%.

Statistical Analysis

An overall test for significance between the 3 groups: HIV-infected subjects with fat redistribution (LIPO), HIV-infected subjects without fat redistribution (NONLIPO), and healthy control (C) subjects was performed for each variable using 1-way analysis of variance (ANOVA). Separate pairwise comparisons using *t* tests were then only performed for those variables that were significant by ANOVA ($P < .05$). The percent of patients with FFA concentrations greater than the upper limit of the normal range was compared between patient groups by χ^2 analysis. Univariate regression analysis was used to determine the relationship between FFA and anthropometric and metabolic parameters. Multivariate regression analysis was performed to determine the independent effects of body composition on FFA and the independent effect of FFA on indices of insulin resistance in this population. Insulin area under the curve (AUC) (measured in μIU/mL) was calculated from the following formula: ([(insulin$_{0min}$ + insulin$_{30min}$)/2] * 30) + ([(insulin $_{30min}$ + insulin$_{60min}$)/2] * 30) + ([(insulin$_{60min}$ + insulin$_{90min}$)/2] * 30) + ([(insulin$_{90min}$ + insulin$_{120min}$)/2] * 30). FFA suppression (measured in mmol/L) was calculated from the following formula: (FFA$_{fasting}$ - FFA$_{120min}$).

Statistical analyses were performed using JMP (Version 3.2.2; SAS Institute, Cary, NC).[24] Statistical significance was determined using 2-tailed tests with a *P* value of .05 or less. Results are presented as mean ± SEM unless otherwise indicated.

RESULTS

Baseline clinical characteristics, including age, weight, BMI, body fat distribution, lipid concentrations, and insulin levels in the LIPO, NONLIPO, and C subjects are shown in Tables 1 and 2. Mean lipodystrophy scores were significantly different between subjects with and without fat redistribution (4.1 ± 0.24 *v* 0.5 ± 0.12, $P < .0001$). Fasting FFA concentrations were significantly increased in the LIPO subjects compared with the NONLIPO and healthy control subjects (Table 2). Fasting FFA concentrations were increased above the normal range (0.1 to 0.6 mmol/L; Wako Chemicals) in 71.9%, 50%, and 40.6% of LIPO, NONLIPO, and C subjects, respectively ($P = .004$, LIPO *v* C; $P = .61$, NONLIPO *v* C). Fasting FFA tended to be increased to a greater relative extent in female LIPO subjects than in male LIPO subjects (0.81 ± 0.04 *v* 0.71 ± 0.03

Table 1. Baseline Characteristics and Comparison of Anthropometric Variables by Gender

	All Subjects				Male Subjects				Female Subjects			
	HIV + Lipodystrophy	HIV + Nonlipodystrophy	Control	*P* Value by ANOVA	HIV + Lipodystrophy	HIV + Nonlipodystrophy	Control	*P* Value by ANOVA	HIV + Lipodystrophy	HIV + Nonlipodystrophy	Control	*P* Value by ANOVA
No.	64	30	32	—	45	20	21	—	19	10	11	—
Age (yr)	41.8 ± 1.03	40.1 ± 0.95	40.6 ± 1.27	.538	42.8 ± 1.18	41.0 ± 1.27	42.7 ± 1.71	.647	39.3 ± 1.99	38.2 ± 1.11	36.7 ± 1.05	.597
Duration HIV (yr)	6.6 ± 0.48	6.7 ± 0.71	—	.850	6.5 ± 0.59	5.6 ± 0.60	—	.354	6.6 ± 0.81	8.9 ± 1.58	—	.163
HIV viral load (copies/mL)	7,298 ± 2,834[D]	26,773 ± 11,327	—	.026	10,126 ± 3,982	17,301 ± 4624	—	.282	698 ± 186[D]	58,347 ± 47,431	—	.038
BMI (kg/m^2)	25.7 ± 0.4	24.4 ± 0.5	24.4 ± 0.3	.059	25.6 ± 0.5	24.6 ± 0.5	24.6 ± 0.5	.262	25.9 ± 1.0	24.0 ± 1.1	24.0 ± 0.3	.250
WHR	0.97 ± 0.01[C,F]	0.89 ± 0.01	0.88 ± 0.01	<.0001	0.97 ± 0.01[C,F]	0.91 ± 0.01	0.90 ± 0.01	<.0001	0.96 ± 0.01[C,F]	0.87 ± 0.02	0.82 ± 0.02	<.0001
Waist circumference (cm)	93.9 ± 1.16[C,E]	87.4 ± 1.36	87.0 ± 1.47	.0002	93.0 ± 1.19	88.6 ± 1.29	90.2 ± 1.55	.070	96.0 ± 2.69[C,D]	84.9 ± 3.14	81.0 ± 2.16	.001
Trunk fat (g)	10,119 ± 531[A,E]	7,413 ± 607	8,016 ± 458	.002	9,392 ± 556[E]	6,836 ± 561	7,876 ± 563	.011	11,840 ± 1140	8,568 ± 1,419	8,271 ± 815	.056
Extremity fat (g)	6,180 ± 421[C,E]	8,316 ± 684	8,541 ± 476	.001	5,147 ± 305[C,E]	6,998 ± 509	7,494 ± 455	<.0001	8,626 ± 1,039	10,953 ± 1,504	10,443 ± 801	.296

NOTE. *P* values determined by *t* test. Results are expressed as mean ± SEM.
A = *P* value < .05, B = *P* value < .01, C = *P* value < .001 *v* respective healthy control subjects.
D = *P* value < .05, E = *P* value < .01, F = *P* value < .001 *v* respective HIV + nonlipodystrophy subjects.

Table 2. Baseline Characteristics and Comparison of Metabolic Variables by Gender

	All Subjects				Male Subjects				Female Subjects			
	HIV + Lipodystrophy	HIV + Nonlipodystrophy	Control	*P* Value by ANOVA	HIV + Lipodystrophy	HIV + Nonlipodystrophy	Control	*P* Value by ANOVA	HIV + Lipodystrophy	HIV + Nonlipodystrophy	Control	*P* Value by ANOVA
No.	64	30	32	—	45	20	21	—	19	10	11	—
Fasting glucose (mg/dL)	92.1 ± 1.52	97.1 ± 4.99	89.1 ± 1.32	.139	94.0 ± 1.73	95.3 ± 3.42	90.4 ± 1.23	.358	87.4 ± 2.88	100.6 ± 13.77	85.9 ± 2.82	.285
120-min OGTT glucose (mg/dL)	133.4 ± 4.88[C,E]	108.8 ± 7.01	94.2 ± 4.84	<.0001	134.4 ± 6.02[C,D]	108.1 ± 8.45	96.9 ± 7.10	.001	131.1 ± 8.42[B]	111.4 ± 10.71[A]	89.0 ± 3.85	.003
Fasting insulin (μIU/mL)	17.5 ± 1.91[C]	12.5 ± 3.24	7.8 ± 0.51	.003	18.3 ± 2.58[A]	13.2 ± 3.54	8.4 ± 0.76	.044	15.6 ± 1.89[B]	5.48 ± 0.65[B]	6.7 ± 0.13	.001
120-min OGTT insulin (μIU/mL)	92.3 ± 11.56[C,E]	27.6 ± 3.46	32.4 ± 6.06	<.0001	88.9 ± 12.8[B,E]	28.5 ± 3.63	35.7 ± 8.27	.001	100.4 ± 25.1	18.2 ± 12.8	26.4 ± 8.27	.064
Insulin AUC (μIU/mL)	9765 ± 922[C,E]	5,099 ± 356	4,943 ± 640	<.0001	9,530 ± 1,063[A,E]	5,183 ± 342	5,768 ± 881	.005	10,374 ± 1885[B]	4,677 ± 1,432	3,443 ± 661	.015
Fasting FFA (mmol/L)	0.74 ± 0.03[B,E]	0.60 ± 0.04	0.59 ± 0.03	.0004	0.71 ± 0.03[D]	0.58 ± 0.04	0.62 ± 0.05	.046	0.81 ± 0.04[C,D]	0.63 ± 0.07	0.54 ± 0.04	.001
120-min OGTT FFA (mmol/L)	0.45 ± 0.01[C,E]	0.39 ± 0.01[C]	0.30 ± 0.01	<.0001	0.46 ± 0.01[C,E]	0.39 ± 0.01[C]	0.30 ± 0.02	<.0001	0.42 ± 0.03[B]	0.36 ± 0.01[B]	0.31 ± 0.01	.018
FFA suppression (mmol/L)	0.29 ± 0.02	0.22 ± 0.05	0.29 ± 0.03	.308	0.25 ± 0.03	0.19 ± 0.05	0.32 ± 0.05	.117	0.39 ± 0.04[A]	0.38 ± 0.09	0.23 ± 0.04	.037
Triglyceride (mg/dL)	349.2 ± 42.36[C,E]	158.5 ± 22.69[C]	73.5 ± 5.58	<.0001	395 ± 56.71[C,E]	152.2 ± 31.18[A]	81 ± 9.38	<.0001	244.3 ± 42.74[B]	171.2 ± 29.06[B]	72.4 ± 9.72	.009

NOTE. *P* values determined by *t* test. Results are expressed as mean ± SEM.
A = *P* value < .05, B = *P* value < .01, C = *P* value < .001 *v* respective healthy control subjects.
D = *P* value < .05, E = *P* value < .01, F = *P* value < .001 *v* respective HIV + nonlipodystrophy subjects.

Table 3. Fasting and 120-Minute OGTT FFA Concentrations in HIV-Infected Patients With Lipodystrophy by Current Medication Status (N = 64)

	PI		NNRTI	
	Yes (N = 45)	No (N = 19)	Yes (N = 18)	No (N = 46)
Fasting FFA (mmol/L)	0.74 ± 0.03	0.75 ± 0.04	0.80 ± 0.04	0.72 ± 0.03
120-minute OGTT FFA (mmol/L)	0.45 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.45 ± 0.02

NOTE. Since all LIPO subjects were on a NRTI, a subanalysis of fasting and 120-minute OGTT FFA levels by NRTI status was not performed. Results are expressed as mean ± SEM. *P* value > .05 for all comparisons.

Abbreviations: PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

mmol/L, $P = .06$). Similarly, fasting triglycerides and 120-minute OGTT FFA were significantly increased in patients with HIV lipodystrophy as compared with their respective nonlipodystrophic and healthy controls (Table 2). Fasting FFA concentration was significantly associated with triglyceride concentration ($r = .38$, $P = .002$) among the HIV-infected subjects with fat redistribution. Fasting FFA concentrations and FFA suppression were both normally distributed among HIV-infected subjects. In contrast, the 120-minute OGTT FFA concentrations were not normally distributed among these same subjects. Log transformation of the 120-minute OGTT FFA concentrations resulted in a normal distribution. In the comparison of the log-transformed FFA data between HIV-infected patients with and without fat redistribution, the difference between the groups remained significant (data not shown).

Among HIV-infected patients with lipodystrophy, there was no significant association between fasting FFA and insulin levels obtained at 0, 30, and 60 minutes during the OGTT ($insulin_{0\ minutes}$, $r = .05$, $P = .708$; $insulin_{30\ minutes}$, $r = .19$, $P = .133$; $insulin_{60\ minutes}$, $r = .22$, $P = .088$). In contrast, there was a significant association between fasting FFA and insulin levels obtained at 90 and 120 minutes during the OGTT ($insulin_{90\ minutes}$, $r = .29$, $P = .019$; $insulin_{120\ minutes}$, $r = .33$, $P = .008$) and between fasting FFA and insulin AUC ($r = .35$, $P = .005$).

Significant differences were observed between the groups in the glucose to insulin ratio at 120 minutes during the OGTT (LIPO *v* NONLIPO *v* C, 2.4 ± 0.19 *v* 5.2 ± 0.67 *v* 4.8 ± 0.53, $P < .0001$ by ANOVA). Both fasting FFA and 120-minute OGTT FFA were inversely associated with the 120-minute OGTT glucose to insulin ratio in HIV-infected subjects with fat redistribution (fasting FFA, $r = .30$, $P = .017$; 120-minute OGTT FFA, $r = .27$, $P = .033$, respectively).

No differences were observed in FFA concentrations in the LIPO group in an analysis based on current protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) use (Table 3). Because all subjects in the LIPO group were receiving nucleoside reverse transcriptase inhibitor (NRTI) therapy, as distinguished from NNRTI therapy, comparisons were not made on NRTI status.

The 120-minute OGTT FFA concentrations were significantly increased in LIPO subjects compared with their respective NONLIPO and healthy controls (0.45 ± 0.01 *v* 0.39 ± 0.01 mmol/L, $P < .001$, LIPO *v* NONLIPO; 0.45 ± 0.01 *v* 0.30 ± 0.01 mmol/L, $P < .0001$, LIPO *v* C). Trunk fat was a significant positive predictor ($P = .04$, r^2 for the model = .16) of the 120-minute OGTT FFA concentration, after controlling for gender, BMI, and extremity fat (Table 4) among the patients in the LIPO group.

In multivariate regression modeling of LIPO subjects, fasting FFA was a significant independent predictor ($P = .027$, r^2 for model = .31) of insulin AUC, controlling for age, gender, BMI, and body composition parameters (trunk and extremity fat) (Table 5). Identical multivariate modeling performed separately in NONLIPO and C subjects did not show any significant relationship between fasting FFA and insulin AUC (data not shown). Among HIV-infected patients with fat redistribution, insulin AUC increased 954 μIU/mL (approximately 10%) for every 0.1 mmol/L increase in fasting FFA concentration.

DISCUSSION

In this study, we demonstrate increased fasting and post-glucose challenge FFA concentrations in HIV-infected subjects with evidence of fat redistribution compared with both HIV-infected subjects without fat redistribution and HIV-negative healthy controls of similar age and BMI. Fasting FFA concentrations were significantly associated with insulin responses to standard glucose challenge, controlling for age, weight, gender, and indices of fat distribution in HIV-infected patients with fat redistribution. An effect of gender on FFA concentrations was observed in the subjects with lipodystrophy, with generally higher fasting FFA concentrations found in female subjects compared with male subjects. In the LIPO group, increased

Table 4. Univariate Analyses and Multivariate Regression Model to Predict 120-Minute OGTT FFA in HIV-Infected Patients With Lipodystrophy ($r^2 = .164$ for multivariate model)

	Univariate Analyses		Multivariate Model		
Variable	*r*	*P* Value	Estimate	95% CI	*P* Value
Age (yr)	.27	.028	0.0029	−0.0003, 0.0061	.072
Gender	.16	.215	−0.0312	−0.0971, 0.0347	.347
BMI (kg/m^2)	.08	.547	−0.0066	−0.0206, 0.0073	.345
Extremity fat by DXA (g)	.11	.387	−0.00001	−0.00002, 0.00001	.244
Trunk fat by DXA (g)	.14	.285	0.00001	0.000001, 0.00003	.040

Table 5. Univariate Analyses and Multivariate Regression Model to Predict Insulin AUC in HIV-Infected Patients With Lipodystrophy (r^2 = .313 for multivariate model)

	Univariate Analyses		Multivariate Model		
Variable	*r*	*P* Value	Estimate	95% CI	*P* Value
Age (yr)	.29	.025	214.5	6.1, 422.9	.044
Gender	.05	.685	1,089.4	−3,433.0, 5,611.8	.631
BMI (kg/m^2)	.21	.104	68.6	−856.1, 993.3	.882
Fasting FFA (mmol/L)	.35	.005	9,542.1	1,133.8, 17,950.5	.027
Extremity fat by DXA (g)	.004	.973	−0.6835	−1.428, 0.0614	.071
Trunk fat by DXA (g)	.29	.023	0.8064	−0.0317, 1.644	.059

trunk fat was a significant independent predictor of FFA concentrations post-glucose challenge.

Our data demonstrate significant increases in FFA and triglyceride concentrations in HIV-infected patients with fat redistribution as compared with HIV-infected subjects without fat redistribution and with healthy control subjects, both of similar age and BMI and provide preliminary evidence that increased FFA may be an important mechanism of insulin resistance in this population. Vigouroux et al[17] demonstrated insulin resistance, glucose intolerance, hypertriglyceridemia, and increased FFA concentrations before and after glucose challenge in 14 HIV-infected patients (4 women, 10 men) with fat redistribution, without direct comparison to age- and BMI-matched control subjects or detailed assessment of body composition. In contrast, Carr et al[6] did not observe a significant difference in FFA concentrations between HIV-infected subjects with and without lipodystrophy (98% of whom were male), but demonstrated increased FFA concentrations in HIV-infected patients receiving PI therapy with abnormal glucose tolerance.[3]

More recently, Mynarcik et al[16] demonstrated normal FFA concentrations in 15 HIV-infected patients with fat redistribution. Using hyperinsulinemic clamp, Mynarcik et al[16] demonstrated that decreased extremity fat and increased soluble type 2 tumor necrosis factor-α receptor, but not FFA, contributed to insulin resistance in this group of patients with normal FFA concentrations. Our study was of a sufficient size to demonstrate that increased FFA predicted hyperinsulinemic response to glucose challenge in HIV-infected patients with fat redistribution. In contrast, no effect of fasting FFA on insulin AUC was seen in HIV-infected patients without fat distribution or among healthy control subjects. Our study suggests that there may be subpopulations of HIV-infected patients with relatively greater increases in FFA concentrations, such as older patients or patients with severe increases in trunk fat.

The mechanism of increased FFA concentrations in HIV lipodystrophy is not known. Increased trunk fat was a significant predictor of FFA response to glucose challenge, but reduced extremity fat did not appear to contribute to either fasting or 120-minute OGTT FFA concentrations. Assessment of trunk fat by DXA combines measurement of both visceral and subcutaneous fat. Prior studies have shown a strong correlation between FFA and visceral fat in HIV-negative subjects.[13,25-27] Visceral fat was not specifically assessed in this study, but has been shown to be significantly increased in the HIV lipodystrophy syndrome and may contribute to the markedly elevated FFA levels in this population. Our cross-sectional data using DXA do not suggest a significant effect of extremity fat, but rather a more important effect of trunk fat in mediating 120-minute FFA concentrations. Further studies are necessary to determine the relative contribution of visceral and subcutaneous abdominal fat to increased FFA in HIV-infected patients with fat redistribution.

Age, like increased trunk fat, was a significant predictor of FFA response to glucose challenge in HIV-infected patients with lipodystrophy. The body fat redistribution that occurs in patients with HIV lipodystrophy parallels the known changes that occur in body fat distribution with normal aging, including increased visceral fat and decreased extremity fat.[5,28]

Although Heijligenberg et al[29] demonstrated increased FFA concentrations in a small study of 6 HIV-infected patients without reported lipodystrophy, our data, in a larger comparison, do not suggest that FFA concentrations are increased as a function of HIV per se. Comparison of gender-matched HIV-infected patients without evidence of lipodystrophy versus HIV-negative control subjects of similar age and BMI showed no difference in fasting FFA levels, although 120-minute OGTT FFA were significantly higher in the NONLIPO versus C subjects.

One factor influencing FFA may be gender. Relatively increased fasting FFA concentrations were observed in female HIV-infected subjects with fat redistribution compared with male HIV-infected subjects with fat redistribution. The sex differences that exist in fasting FFA concentrations in subjects with HIV lipodystrophy are not unlike those found in other populations.[30,31] Byrne et al[30] demonstrated that FFA concentrations were statistically greater in women compared with men. In our study, trunk fat was significantly greater in female HIV-infected patients with fat redistribution as compared with male HIV-infected patients with fat redistribution, perhaps contributing to the observation of increased FFA in these female patients.

We investigated FFA pre-and post-OGTT to determine the degree to which glucose (and by extension, insulin) suppresses lipolysis. The 120-minute OGTT FFA concentrations remained increased in the HIV-infected subjects with fat redistribution as compared with both HIV-infected subjects without fat redistribution and HIV-negative control subjects. The degree of FFA suppression (change from baseline) during glucose challenge was similar in all 3 groups despite a 2-fold greater insulin response among the HIV-infected patients with fat redistribution (combined analysis including male and female subjects).

Taken together, these data suggest increased basal lipolytic rates in HIV-infected patients with fat redistribution and potential resistance to the action of insulin with respect to suppression of lipolysis in HIV-infected subjects with lipodystrophy. Indeed, preliminary data using stable isotope infusion suggest increased basal rates of lipolysis in HIV lipodystrophy,[32] but further studies investigating the sensitivity of lipolysis to suppression with insulin have not been performed.

Our data suggest that fasting FFA is a strong predictor of insulin AUC and reduced glucose to insulin ratios in response to glucose challenge. Randle et al[33] proposed a causal relationship between elevated FFA concentrations and impaired glucose tolerance, suggesting that increased fat oxidation leads to insulin resistance by interfering with glucose oxidation. Still others have shown that FFA limits insulin-mediated suppression of hepatic glucose output or promotes gluconeogenesis.[10-15] In recent years, the mechanism originally suggested by Randle et al[33] has been refined. With the use of a hyperinsulinemic-euglycemic clamp and lipid infusion, Dresner et al[9] have shown that the insulin resistance induced by free fatty acids results from inhibition of glucose transport activity.

In conclusion, we have shown increased FFA concentrations in HIV-infected subjects with fat redistribution, suggesting increased lipolytic rates. Our data demonstrate that increased trunk fat may contribute to increased lipolysis in this population. Moreover, we show for the first time that basal FFA concentrations are a strong independent predictor of hyperinsulinemia in HIV lipodystrophy. Further studies are necessary to determine lipolytic rates, the contribution of visceral adiposity, and the role played by increased FFA in mediating the insulin resistance in this population.

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