Lipodystrophy in Human Immunodeficiency Virus Patients Impairs Insulin Action and Induces Defects in β-Cell Function

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The pathophysiology of insulin resistance in human immunodeficiency virus (HIV)-associated lipodystrophy syndrome (HALS) is not fully clarified. We investigated 18 men with HALS and 18 HIV-positive males without lipodystrophy (control subjects). Duration and modality of antiretroviral therapy were similar between study groups. A hyperinsulinemic euglycemic clamp showed an impaired glucose disposal rate (GDR) in HALS patients (5.6 *v* 8.3 mg glucose/min · kg_{FFM}, *P* = .0006). As demonstrated by indirect calorimetry, HALS patients showed an impaired nonoxidative glucose metabolism (NOGM, 2.2 *v* 4.2, *P* = .006), whereas levels of basal and insulin-stimulated oxidative glucose metabolism (OGM) (2.4 *v* 2.3, *P* = .55, and 3.3 *v* 4.0, *P* = .064, respectively) were not significantly different between groups. Despite comparable total fat masses, dual energy x-ray absorptiometry (DEXA) scans showed that the percentage of limb fat (ie, peripheral-fat-mass/[peripheral-fat-mass + trunk-fat-mass] · 100%) was reduced in HALS patients (36% *v* 46%, *P* = .0002). Multiple linear regression analysis indicated that percentage of limb fat explained 53% of the variability of GDR and 45% of the variability of NOGM in HALS patients. In HALS patients, leg fat mass correlated positively with NOGM (*r* = .51, *P* < .05), whereas abdominal fat mass and NOGM did not correlate (*P* = .91). Analyzing the relationship between first phase insulin secretion and insulin sensitivity, 6 HALS patients compared with none of the control subjects exhibited impaired insulin secretion (*P* < .05). Our data suggest that fat redistribution independently of antiretroviral therapy is highly related to insulin resistance in HALS patients. Furthermore, in HALS patients, impaired glucose metabolism most likely relates to decreased NOGM and to defects in β-cell function.

RECENTLY, A SYNDROME of fat redistribution in human immunodeficiency virus (HIV)-affected patients denoted HIV-associated lipodystrophy syndrome (HALS) has been acknowledged. The syndrome has been shown to be associated with insulin resistance.[1-4] The definition of HALS, however, is still a matter of debate.[5,6] In a number of case control studies, HALS has been defined as centrifugal fat loss simultaneous with centripetal fat reorganization, ie, loss of subcutaneous fat in the face, buttocks, and a fat loss from extremities and a gain of visceral abdominal fat. More rarely, the syndrome has been characterized by a fat pad accumulation in the neck region, in the mammae or by subcutaneous lipomathosis. In some studies, HIV patients with merely lipoatrophy, and patients with merely central fat gain, have been accepted as meeting the criteria of HALS, rendering HIV wasting and age-dependent abdominal fat accumulation as confounders when interpreting the results.[1,4,6-10] In 5 major prevalence studies of lipodystrophy, a significant fraction of the HALS patients undergoing highly active antiretroviral therapy (HAART) presented a combined fat loss and fat accumulation, ie, mixed lipodystrophy (38% to 54%). Nevertheless, an important fraction was either diagnosed by peripheral lipoatrophy (25% to 34%) or central obesity (13% to 34%).[6,10-13]

The development of HALS has been associated with the treatment duration of protease inhibitors (PI).[1,7] Recently, however, the syndrome has also been recognized in HIV patients exclusively treated with nucleoside reverse transcriptase inhibitors (NRTI).[9,14] Furthermore, in a study including HALS patients with mixed NRTI and PI regimens, multiple regression analysis indicated that NRTI treatment may be even more strongly correlated to lipodystrophy than PI treatment.[10]

In normal-weight male HALS patients, insulin resistance, as estimated by fasting insulin and homeostasis model assessment insulin resistance index (HOMA-IR), has been associated with fat distribution.[1,10] Using the hyperinsulinemic clamp technique, it was found that insulin action and relative amount of limb fat correlated across the study groups (HALS, HIV-lipodystrophy negative, and HIV-negative healthy control subjects, respectively), but not within the individual groups.[15] In a study including PI-treated HALS patients, PI-treated nonlipodystrophic HIV patients, and nonlipodystrophic HIV PI naive patients, respectively, associations of peripheral fat mass and of trunk fat mass versus insulin action across the study groups were reported.[16] In that study, the frequently sampled intravenous glucose tolerance test (FSIGTT) and the minimal model approach were used to calculate insulin sensitivity. Interestingly, a recent report including 6 nondiabetic HALS patients and 6 healthy subjects reported major impairments in insulin stimulated nonoxidative glucose metabolism (NOGM) in the HALS group.[17] Such defects in NOGM have been reported in overt type 2 diabetes mellitus.[18]

The aim of the present investigation was to compare insulin action and β-cell function in a group of male HALS patients with a group of male HIV patients not reporting abnormal fat redistribution. The patients were examined by indirect calorimetry, a hyperinsulinemic euglycemic clamp, an intravenous

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glucose tolerance test (IVGTT), and dual energy x-ray absorptiometry (DEXA), respectively.

MATERIAL AND METHODS

Subjects

The patients were recruited consecutively from the outpatient clinic at the Department of Infectious Diseases, Hvidovre University Hospital, Copenhagen, Denmark from September 1999 until June 2000. Male patients older than 18 years, with positive HIV-1 antibody test receiving more than 12 months of HAART, who complained of changes in fat distribution, were informed about the study. The patients were asked to fill in a questionnaire, which included 9 criteria of lipodystrophy (loss of fat in face, arms, legs, buttocks, gain of fat at abdomen, trunk, more exposed veins, fat pads in the neck region, and lipomas). A trained physician performed all of the physical examinations (O.A.) (examination for lipoatrophy in face, extremities, buttocks, abdominal obesity, buffalo hump, and lipomas). The patient had to report at least 1 criteria of lipodystrophy and present at least 1 of 6 signs of lipodystrophy to be categorized as a HALS patient. As for control subjects, a questionnaire about lipodystrophy, as well as the physical examination for signs of lipodystrophy, had to be negative to include a patient. Criteria for exclusion were diabetes mellitus,[19] chronic disease other than HIV, an acquired immune deficiency syndrome (AIDS)-related episode or acute infection within the latest 3 months, weight loss or gain above 4 kg within 6 months, and treatment with antilipid or antidiabetic drugs. None of the subjects was engaged in competitive sports or treated with drugs known to influence glucose metabolism (except for HAART[20]). Reasons for exclusion among HALS patients were diabetes mellitus (2 patients) and chronic disease other than HIV (1 patient with a myocardial infarction). We excluded from the study 1 HALS patient and 2 control subjects who missed the DEXA scanning. Eighteen HALS patients and 18 HIV patients without lipodystrophy were evaluated by metabolic and body composition measurements as described below. 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.

Experiments

Patients were advised to not alter their normal diet and to not perform strenuous physical exercise for 3 days before metabolic assessments. The patients were admitted to the clinical research center after absence from HIV medication for 18 hours and an overnight 12-hour fast. They were studied in the supine position at room temperature (25°C). A catheter was inserted in the antecubital vein of each arm. One catheter was used for sampling and the other catheter for infusion. After obtaining basal blood samples, an indirect calorimetry lasting 30 minutes was performed for basal glucose oxidation measurements. To characterize the first phase insulin response, an FSIGTT (duration, 30 minutes) was performed. Thereafter, a needle biopsy from the vastus lateralis was taken (ie, for later analysis of mitochondrial DNA content). The FSIGTT and needle biopsy were followed by a hyperinsulinemic euglycemic clamp (120 minutes). During the last 30 minutes of the clamp period (the insulin-stimulated steady state period), another indirect calorimetry was performed to measure insulin-stimulated glucose oxidation. On a following day, DEXA scan was performed to estimate body composition.

FSIGTT and hyperinsulinemic euglycemic clamp. Baseline blood samples were drawn at −4, −2, and 0 minutes. At 0 minute, a bolus of 300 mg glucose/kg body weight of 20% glucose was injected intravenously over 60 seconds. Venous blood was sampled at 2, 4, 6, 8, 10, 15, 20, and 30 minutes for measurement of glucose, insulin, and C-peptide. Immediately after the last FSIGTT sampling, we started insulin infusion, performing a squared priming (0 to +9 minutes) with stepwise decline in the insulin (Actrapid; Novo Nordisk A/S, Bagsvaerd, Denmark) infusion rate every third minute, reducing the insulin infusion rate from 100 to 80 to 60 to 40 mU/m^2 × min. Thereafter (+9 to 120 minutes), the insulin infusion rate was fixed at 40 mU/m^2 × min. Plasma glucose concentration was maintained constantly at euglycemia (5 mmol/L) using a variable glucose infusion of 20% glucose.[21] During the clamp, glucose levels were monitored every 5 minutes, and the insulin levels were assessed every 15 minutes. The average glucose infusion rate measured during the last 30 minutes of the clamp was considered to represent whole body glucose disposal, a marker of peripheral insulin action.

Indirect calorimetry. A ventilated canopy was placed over the subject's head (Deltatrac II Metabolic Monitor; Datex, Helsinki, Finland), and continuous gas exchange was determined. Inspired and expired air was analyzed for oxygen concentration using a paramagnetic differential oxygen sensor and for carbon dioxide using an infrared carbon dioxide sensor. Oxygen consumption and carbon dioxide production were recorded and calculated each minute. After an equilibrium period of 10 minutes, the average gas exchange over the two 30-minute steady states periods (basal and insulin stimulated) were used to calculate rates of glucose oxidation as previously described.[22] Glucose turnover rates are expressed in mg/min/kg fat-free mass (FFM) and presented throughout in this report as mean values of the 30-minute steady-state period.

Body composition and anthropometry. Body composition was estimated by DEXA scanning (Norland Medical System XR-36, Fort Atkinson, WI) using software version 2.1.0. A whole-body scan was performed to estimate the amount of fat in the trunk and extremities. The trunk was defined as the region including the chest, abdomen, and pelvis excluding neck and head. The proximal limitations of the leg regions were placed through the hip joints at an angle of approximately 45° and for the arm regions vertically through the shoulder joints. Two regions of interest (ROI) were defined to measure abdominal tissue distribution as previously described.[23,24] The area between horizontal lines at the levels of the xiphoid process and the iliac crest was denoted "abdominal PX-IC." The area between horizontal lines at the levels of the top of the second and bottom of the fourth lumbar vertebra was denoted "abdominal L2-L4." Peripheral fat mass was defined as the sum of arm and leg fat masses. In our hands, DEXA scans have shown precision of 1% for FFM, 3% for total fat mass, 4% for trunk fat mass, and 5% for extremity fat mass. The DEXA scans were performed in random order, and the operator who performed the analysis of body composition was unaware of the assignments of patients to study groups.

Body weight and height was measured on a calibrated scale. Waist circumference was measured at the level of the umbilicus while the subject was standing and after a normal expiration. Hip circumference was measured in the horizontal plane at the level of the maximal extension of the buttocks. Weight, height, waist circumference, and hip circumference were measured in duplicate by the same investigator, and mean values were noted.

Assays. The whole blood glucose levels were determined pairwise on 2 calibrated HemoCue B-Glucose Analyzers (HemaCue AB, Angelholm, Sweden) with an intra-analyzer coefficient of variation (CV) of 3.5% and an interanalyzer CV of 3.3%. Plasma glucose was calculated using the equations of Fogh-Andersen,[25] filling in data on whole blood glucose (the mean of the pair of measurements) and blood hematocrit (HCT). Plasma insulin and C-peptide blood samples were centrifuged immediately at 4°C and stored at −80°C for later analysis. Plasma insulin and C-peptide concentrations were determined by 1235 AutoDELPHIA automatic immunoassay system (Wallac Oy, Turku, Finland). The insulin assay had a detection limit of appoximately 3 pmol/L. Cross-reactivity with intact proinsulin was 0.1%, 0.4% with 32-33 split proinsulin, and 66% with 64-65 split proinsulin, intra-assay

CV of 4.5%, and interassay CV of 7%. Detection limit of the C-peptide assay was 5 pmol/L. Cross-reactivity with intact proinsulin was 51%, 35% with 32-33 split proinsulin, and 92% with 64-65 split proinsulin, no detectable cross-reactivity with insulin, intra-assay variation of 5%, and interassay variation of 8%. Plasma free fatty acids were determined using an enzymatic colorimetric method (Wako C test kit, Wako Chemicals, Neuss, Germany) with an interassay CV of 5%. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and serum-triglycerides were determined by reflection photometry (Ortho-Clinical Diagnostics kit, Raritan, NJ.) with interassay CV of 2%, 8%, and 2.5%, respectively. Serum cortisol was determined by the radioimmunoassay (RIA) method (Diagnostic System Laboratories, Houston, TX) with an interassay CV of 9%. CD4 count determination (flow cytometry, Becton-Dickinson FACscan, BD, Franklin Lakes, NJ; interassay CV of 7%) and viral load determination (Roche Amplicor and Roche Amplicer Ultrasensitive assay with a detection limit of 20 copies/mL plasma; Roche, Basel, Switzerland) met the requirements of interlaboratory quality control.

Calculations

The relative amount of peripheral fat compared with trunk fat, the percentage of limb fat, was calculated as previously described[15] (ie, peripheral-fat-mass/[peripheral-fat-mass + trunk-fat-mass] · 100%). The regional fat masses were normalized by body weight (eg, leg fat (%) = leg fat mass/body weight [BW] × 100%). The insulin sensitivity index (Si) was calculated as the mean glucose infusion rate during the clamp steady state period divided by the clamp steady state plasma insulin and the clamp steady state glucose concentration and the body weight or the FFM (unit: $L^2 \cdot \mu mol^{-1} \cdot min^{-1} \cdot kg^{-1}$). The IV glucose tolerance during FSIGTT was defined as the slope of the glucose curve between 8 minutes and 30 minutes.

As a measure of β-cell function, the acute insulin (first phase) response to glucose ($AIRg_{0\text{-}10}$) was calculated by means of the trapezoidal rule, as total incremental area under the curve, 0 to 10 minutes after the glucose bolus injection. In subjects with normal glucose tolerance, $AIRg_{0\text{-}10}$ will increase as Si is reduced. The product of these 2 parameters is approximately a constant, named disposition index ($DI = Si \cdot AIRg_{0\text{-}10}$). Thus, as suggested by Bergman et al[26] in 1981 and confirmed by Kahn et al[27] in 1993, the relationship between Si and $AIRg_{0\text{-}10}$ is a hyperbola. Therefore, a failure to fit a hyperbolic relationship between Si and $AIRg_{0\text{-}10}$ might be due to an insufficient adaptation of the pancreatic β cells to the concomitant insulin sensitivity. The following strategy was designed to identify patients with reduced capacity of β-cell adaptation. After sorting the patients according to increasing DI, it was analyzed whether omission of those with the lowest DI lead to an improved fit of the hyperbolic relationship between Si and $AIRg_{0\text{-}10}$ such that it became statistically significant and significantly better than the fit with all patients. In line with the work of Kahn et al,[27] we also calculated the relationship between fasting insulin and Si, testing in our patients that this relationship would fit a hyperbola. It should be noted that in the original report by Kahn et al,[27] Si was calculated by the minimal model approach, and that the validity of our measures to that described by Kahn et al has not been investigated.

Prehepatic insulin secretion was calculated from plasma C-peptide measurements using the ISEC (Insulin SECretion) computer program.[28] The model is based on the assumptions that insulin and C-peptide are cosecreted in an equimolar fashion by the pancreas and that C-peptide is not cleared by the liver. ISEC has been validated to calculate prehepatic insulin secretion during FSIGTT[29,30] and has been applied to calculate prehepatic insulin secretion profiles during meal tolerance test, hyperinsulinemic euglycemic clamp, and during basal conditions.[28] The calculated prehepatic insulin response (see Fig 3) to IV glucose for each study group showeded that practically all insulin was secreted within the first 6 minutes. We denoted this amount of insulin the acute prehepatic first phase insulin secretory response ($AIRs_{0\text{-}6}$). $AIRs_{0\text{-}6}$ was calculated by means of the trapezoidal rule as total incremental area under the curve, 0 to 6 minutes after the glucose bolus injection. $AIRs_{0\text{-}6}$ was expressed as $pmol \cdot kg^{-1}$ and for 'whole body' response, as pmol.

Statistical Analysis

Data are presented as means ± SEM and as medians and ranges when distributions were skewed. The *t* test was used to compare the distribution of parameters between cases and control subjects. Pearson correlation coefficent (*r*) was applied for measurements of associations between variables within each study group and for pooled study groups. When data distribution was skewed, data were log transformed before applying a *t* test and before calculation of a correlation coefficient. A stepwise multiple linear regression method with a lax criterion for inclusion, ie, $P < .3$ was applied to detect the most important among many putative predictors of insulin action. Calculations were performed by SPSS (version. 10.0.7; SPSS, Chicago, IL). Two-sided *P* values less than .05 were considered significant.

RESULTS

Patients, Lipodystrophy, and Treatment

Eighteen HIV-1 patients with self-reported and physician-approved lipodystrophy (HALS patients) were included as cases. As control subjects, we included 18 HIV-1 positive patients not reporting lipodystrophy and by physician confirmed without signs of lipodystrophy. None of the HALS patients had first-degree relatives with type 2 diabetes mellitus, but two of the control subjects did. All patients were Caucasians, except 2 black Africans (control subjects). None of the patients were IV drug users. Six HALS patients and 9 control subjects had a prior AIDS defining disease. Seventeen cases reported mixed lipodystrophy, ie, loss of facial fat (n = 13) and loss of fat in extremities (n = 17) with concomitant accumulation of central fat mass (n = 17). One case patient had multiple subcutaneous lipomas as the only sign of lipodystrophy.

Seventeen patients in the HALS group had taken PI for 12 to 58 months. In the control group, 17 patients had been treated with PI for 5 to 54 months (Table 1). All patients had received NRTI treatment, HALS patients, 13 to 111 months; control subjects, 13 to 97 months (Table 1). There was no significant difference between HALS patients and control subjects in the duration of treatment with PI and NRTI. Table 2 presents the current treatment modality, which appeared to be balanced between study groups.

Anthropometry, Fat Distribution, Immunology, and Blood Variables

Anthropometric data are presented in Table 1. HALS patients were slightly older than control subjects. HALS patients were also slightly heavier than control subjects, which could be ascribed to an increased lean body mass in the former group. The study groups exhibited similar total fat masses and peripheral fat masses. Measures of trunk fat mass and abdominal fat mass were increased in HALS patients as were waist circumference and waist-to-hip ratio (WHR). The fat tissue was differently distributed in HALS patients compared with control subjects demonstrated by a parallel displacement of regression

Table 1. Characteristics of Study Groups

	HALS Patients	Controls	*P* Value
No. and sex	18 males	18 males	
Age (yr)	50 (2)	43 (2)	.02
Body mass index (kg/m^2)	24.7 (0.6)	22.5 (0.8)	.04
Lean mass (kg)	60.6 (2.1)	54.4 (1.7)	.04
Total fat mass (kg)	16.4 (1.5)	13.0 (1.5)	.12
Peripheral fat mass (kg)	5.5 (0.6)	5.4 (0.6)	.89
Arm fat mass (kg)	1.7 (0.2)	1.5 (0.2)	.56
Leg fat mass (kg)	3.9 (0.4)	3.9 (0.5)	.79
Trunk fat mass (kg)	9.8 (1.0)	6.7 (0.9)	.02
Abdominal fat mass (kg) L2-L4	3.0 (0.3)	1.6 (0.3)	.0008
Abdominal fat mass (kg) PX-IC	5.6 (0.5)	3.0 (0.5)	.001
Waist circumference (cm)	96 (2)	86 (2)	.005
Waist-to-hip ratio	1.01 (0.01)	0.92 (0.01)	.0002
Total fat (%)	20.1 (1.3)	17.7 (1.6)	.25
Peripheral fat (%)	6.8 (0.6)	7.4 (0.7)	.48
Leg fat (%)	4.7 (0.4)	5.3 (0.6)	.57
Trunk fat (%)	12.2 (0.8)	9.1 (1.1)	.03
Abdominal fat (%) L2-L4	3.7 (0.2)	2.1 (0.3)	.0003
Abdominal fat (%) PX-IC	6.8 (0.4)	4.0 (0.6)	.0006
Percentage of limb fat (= peripheral fat mass/ [peripheral fat mass + trunk fat mass] × 100%)	35.4 (1.3)	46.1 (1.8)	.0001
Duration of HIV infection (mo)	99 (14)	72 (11)	.23
Duration of NRTI treatment (mo)	47 (7)	42 (6)	.56
Duration of PI treatment (mo)	32 (4)	25 (4)	.14
Prior aids defining disease (n)	6	9	
Family history of diabetes mellitus (n)	0	2	
Smoking habits*	1.0 (0.2)	1.3 (0.2)	.40
CD4 cells (× 10^3/mL)	427 (45)	352 (46)	.20
CD4 nadir	130 (24)	123 (32)	.67
CD4 absolute increase	297 (38)	229 (32)	.21
CD4/CD8	0.39 (0.04)	0.41 (0.05)	.99
CD4/CD8 nadir	0.16 (0.03)	0.19 (0.04)	.72
HIV RNA (copies/mL)	<20 (<20-12,700)	<20 (<20-1,590)	.39
HIV RNA peak (copies/mL)	174,008 (<20-1,558,000)	150,000 (7,400-1,404,000)	.79
Fasting plasma glucose (mmol/L)	5.0 (0.2)	4.9 (0.1)	.41
Fasting plasma insulin (pmol/L)	89 (13)	41 (6)	.0008
Fasting plasma C-peptide (pmol/L)	1,146 (128)	650 (75)	.002
GDR (mg/min × kg_{FFM})	5.6 (0.5)	8.3 (0.5)	.006
NOGM (mg/min × kg_{FFM})	2.2 (0.4)	4.2 (0.5)	.006
Fasting OGM (mg/min × kg_{FFM})	2.5 (0.2)	2.3 (0.2)	.55
Insulin-stimulated OGM (mg/min × kg_{FFM})	3.3 (0.2)	4.0 (0.3)	.06
NOGM/GDR × 100%	34 (5)	49 (4)	.02
OGM/GDR × 100%	66 (5)	51 (4)	.02
Si (L × L/(μmol × min × kg_{BW})	9.6 (1.3)	18.8 (1.8)	.001
Si (L × L/(μmol × min × kg_{FFM})	12.7 (1.7)	23.6 (2.1)	.001
$AIRg_{0\text{-}10}$ (pmol/L)	2,437 (432-20,298)	1,675 (529-6,886)	.16
$AIRs_{0\text{-}6}$ (pmol)	6,096 (680-30,921)	5,613 (1,531-16,056)	.35
Di (Si × $AIRg_{0\text{-}10}$) (mL/min × kg_{BW})	39 (10)	44 (7)	.73
Si × $AIRs_{0\text{-}8}$ (L × L/min × kg_{BW})	0.091 (0.020)	0.128 (0.020)	.24
Plasma alanine amino transferase (U/L)	47 (6)	37 (4)	.48
Plasma cortisol (nmol/L)	328 (19)	406 (34)	.06
Free fatty acids (mmol/L)	0.63 (0.06)	0.50 (0.06)	.14
Total plasma cholesterol (mmol/L)	6.3 (0.4)	5.0 (0.2)	.005
Plasma HDL cholesterol (mmol/L)	1.01 (0.11)	1.04 (0.07)	.56
Plasma triglyceride (mmol/L)	2.7 (0.8-16.4)	1.9 (0.7-7.4)	.21

NOTE. Mean (SEM), median (range). Total fat (%) indicates total fat mass normalized for body weight, ie, total fat mass/body weight × 100%.

Abbreviations: L2, lumbal vertebra no. 2; PX, xiphoid process; IC, iliac crest; GDR, glucose disposal rate; OGM, oxidative glucose metabolism; NOGM, nonoxidative glucose metabolism; FFM, fat-free mass; BW, body weight; DI, disposition index; Si, insulin sensitivity; $AIRg_{0\text{-}10}$, acute posthepatic insulin response from 0 to 10 minutes after an IV glucose bolus; $AIRs_{0\text{-}6}$, acute prehepatic secretory insulin response from 0 to 6 minutes after an IV glucose bolus.

*Smoking habits. All smokers smoked nothing but cigarettes; 0 cigarettes per day scored 0, 1 to 10 cigarettes per day scored 1, 11 or more cigarettes per day scored 2.

Table 2. Current Antiretroviral Treatment

Generic Name	Total (n = 36)	Controls (n = 18)	HALS (n = 18)	HALS IB (n = 6)	HALS NB (n = 12)
Any NRTI	35	17	18	6	12
Zidovudin	18	7	9	4	5
Didanosin	3	2	1	1	0
3TC, Lamivudin	30	15	15	5	10
D4T, Stavudin	16	6	10	3	7
Abacavir	1	0	1	0	1
Any non-NRTI	7	6	1	0	1
Nevirapine	4	4	0	0	0
Efavirenz	3	2	1	0	1
Any PI	32	16	16	5	11
Nelfinavir	8	4	4	3	1
Saquinavir	5	2	3	1	2
Ritonavir	11	7	4	1	3
Indinavir	12	4	8	2	6

NOTE. The number of patients taking a specific antiretroviral drug in each study group, in the group of HALS patients with relatively impaired β-cell function (IB, n = 6), and the group of HALS patients with relatively normal β-cell function (NB, n = 12)

lines when trunk fat was plotted against peripheral fat (Fig 1). Thus, the percentage of limb fat was significantly reduced in HALS patients compared with patients without lipodystrophy (36% *v* 46%; $P = .0002$).

The duration of HIV-1 infection, the number of CD4 cells, and the viral load were balanced between HALS patients and control subjects (Table 1). Similarly, the degree of immunologic restitution, as measured by relative and absolute increase in number of CD4 cells, the ratio of CD4 to CD8 and their nadirs, and decrease in HIV RNA were not different between groups. Total cholesterol was increased in HALS patients, whereas HDL cholesterol and plasma triglycerides were similar.

Insulin Action and Fat Distribution

The fasting insulin level was twice as high in HALS patients compared with control subjects despite normoglycemia in both groups (Table1). During the euglycemic clamp the HALS patients exhibited a 33% reduction in glucose disposal rate (GDR) compared with control subjects. The insulin resistance in HALS patients was primarily explained by a 48% reduction in insulin-stimulated NOGM. In HALS patients compared with control subjects, the insulin-stimulated oxidative glucose metabolism (OGM) tended to be significantly reduced. The fasting OGM was similar in the study groups. The ratio of OGM to GDR was increased in HALS patients. The steady state clamp insulin level in HALS patients compared with control subjects was increased (535 ± 35 pmol/L *v* 392 ± 16 pmol/L, $P < .001$). Calculating the Si, therefore, extended the impression of severe insulin resistance among HALS patients compared with control subjects (9.6 ± 1.3 $L^2 \cdot \mu mol^{-1} \cdot min^{-1} \cdot kg_{BW}^{-1}$ *v* 18.8 ± 1.8, $P < .001$).

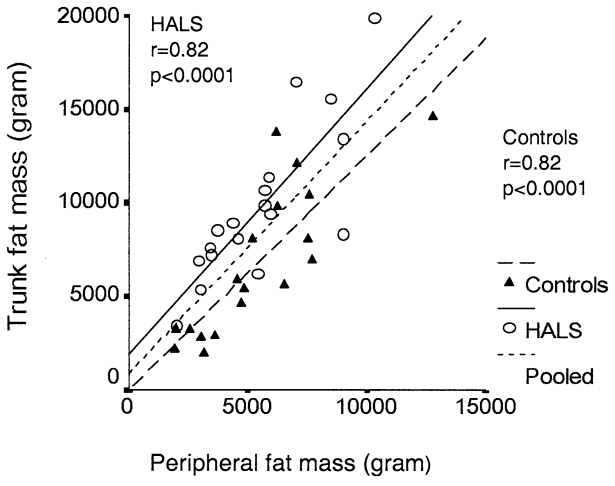

Fig 1. Correlation between trunk fat mass and extremity fat mass in HALS patients (○, regression lines marked by solid line) and in control subjects (▲, regression lines marked by dotted line). The regression line for the pooled data study groups is indicated.

In both study groups GDR, Si, and NOGM, respectively, significantly correlated with percentage of limb fat (Fig 2). Notably, the regression lines for each study group were almost overlying (ie, slopes and intercepts were similar between groups).

Table 3 presents for each study group and for the pooled study groups Pearson correlation coefficients (*r*) between insulin action and a number of possible predictors for the variables, GDR and NOGM. In each study group and for the pooled study groups in this univariate analysis, percentage of limb fat appeared to be the strongest predictor of GDR and NOGM. Notably, among HALS patients, NOGM correlated positively with leg fat (%) ($P = .02$), whereas no such correlation was found between NOGM and abdominal fat L2-L4 (%) ($P = .51$). These correlation were contrasted by the findings in controls subjects of no correlation between NOGM and leg fat (%) ($P = .61$) and a negative correlation between NOGM and abdominal fat L2-L4 (%) ($P = .02$). Other significant associations between predictors of GDR and NOGM are shown in Table 3. It should be noted that age did not correlate with percentage of limb fat in any of the study cohorts.

Multiple Regression Analysis

A multiple linear regression analysis was performed to determine significant independent predictors of GDR and NOGM

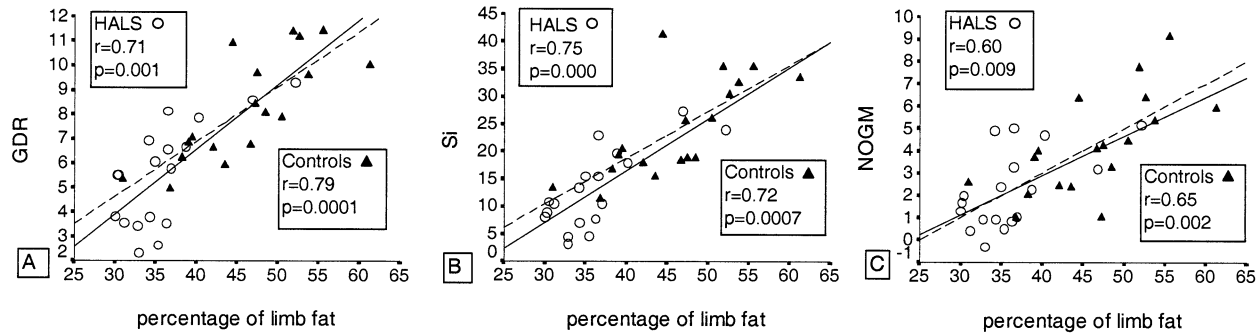

Fig 2. Correlation between measures of insulin action and percentage of limb fat in HALS patients (○, regression lines marked by solid line and in control subjects (▲, regression lines marked by dotted line). (A) Plot of GDR and percentage of limb fat. (B) Plot of Si and percentage of limb fat. (C) Plot of NOGM and percentage of limb fat. The units of GDR and NOGM are mg/glucose/min · kg_{FFM}. The unit of Si is $L^2 \cdot \mu mol^{-1} \cdot min^{-1} \cdot kg^{-1}$.

in each study group and in the pooled cohort. The following putative predictors were included in the analysis: percentage of limb fat, total fat (% and kg), peripheral fat (% and kg), leg fat (% and kg), trunk fat (% and kg), abdominal fat L2-L4 (% and kg), abdominal fat PX-IC (% and kg), WHR, waist circumference, body mass index (BMI), age, duration of HIV, duration on PI, duration on NRTI, p-free fatty acids, total p-cholesterol, p-HDL cholesterol, and p-triglyceride. In HALS patients, control subjects, and the pooled cohort, the percentage of limb fat showed the most important predictor, explaining the variations of GDR and NOGM (HALS: $R^2 = 53\%$ and $R^2 = 45\%$; control subjects: $R^2 = 60\%$ and $R^2 = 42\%$; pooled: $R^2 = 70\%$ and $R^2 = 56\%$,

Table 3. Pearson Correlation Coefficients Between the Dependent Variable GDR and the Dependent Variable NOGM Versus a Number of Putative Predictor Variables

	HALS Patients (n = 18)		Control Subjects (n = 18)		Pooled Study Groups (n = 36)	
	GDR	NOGM	GDR	NOGM	GDR	NOGM
Percentage of limb fat	0.71†	0.60†	0.78‡	0.65†	0.82‡	0.72‡
Total fat (%)	0.11	0.30	−0.59*	−0.46	−0.34*	−0.24
Total fat (kg)	0.01	0.28	−0.60†	−0.41	−0.39*	−0.21
Peripheral fat (%)	0.42	0.52*	−0.29	−0.23	0.07	0.10
Peripheral fat (kg)	0.27	0.48*	−0.37	−0.24	−0.07	0.04
Leg fat (%)	0.45	0.55*	−0.19	−0.13	0.13	0.16
Leg fat (kg)	0.31	0.51*	−0.28	−0.15	−0.01	0.10
Trunk fat (%)	−0.12	0.12	−0.71†	−0.55*	−0.55†	−0.41*
Trunk fat (kg)	−0.15	0.15	−0.70†	−0.49*	−0.53†	−0.34*
Abdominal fat L2-L4 (%)	−0.38	−0.15	−0.75‡	−0.54*	−0.71‡	−0.55†
Abdominal fat L2-L4 (kg)	−0.33	−0.03	−0.73†	−0.49*	−0.65‡	−0.45†
Abdominal fat PX-IC (%)	−0.30	−0.03	−0.73†	−0.52*	−0.68‡	−0.51†
Abdominal fat PX-IC (kg)	−0.26	0.06	−0.71†	−0.48*	−0.63‡	−0.42*
Waist-to-hip ratio	−0.31	−0.27	−0.64†	−0.65†	−0.64‡	−0.59†
Waist circumference	−0.24	−0.02	−0.61†	−0.43	−0.59†	−0.41*
Body mass index	−0.36	−0.11	−0.48*	−0.23	−0.52†	−0.31
Age (yr)	−0.50*	−0.52*	0.08	−0.09	−0.33*	−0.38*
Duration of HIV	−0.24	−0.08	0.40	0.31	−0.09	−0.01
Duration on PI	0.23	0.25	0.65†	0.46	0.23	0.21
Duration on NRTI	−0.10	−0.10	0.41	0.20	0.07	0.01
Free fatty acids	−0.30	−0.32	−0.08	0.02	−0.28	−0.22
Total plasma cholesterol	−0.21	−0.26	−0.48*	−0.52*	−0.45†	−0.46†
Plasma HDL cholesterol	0.16	0.00	0.22	0.04	0.17	0.03
Plasma triglyceride	−0.17	−0.12	0.01	−0.16	−0.22	−0.13

NOTE. "−" indicates a negative correlation.

Abbreviations: GDR, glucose disposal rate; NOGM, nonoxidative glucose metabolism; PI, protease inhibitor, NRTI, nucleoside reverse transcriptase inhibitors.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

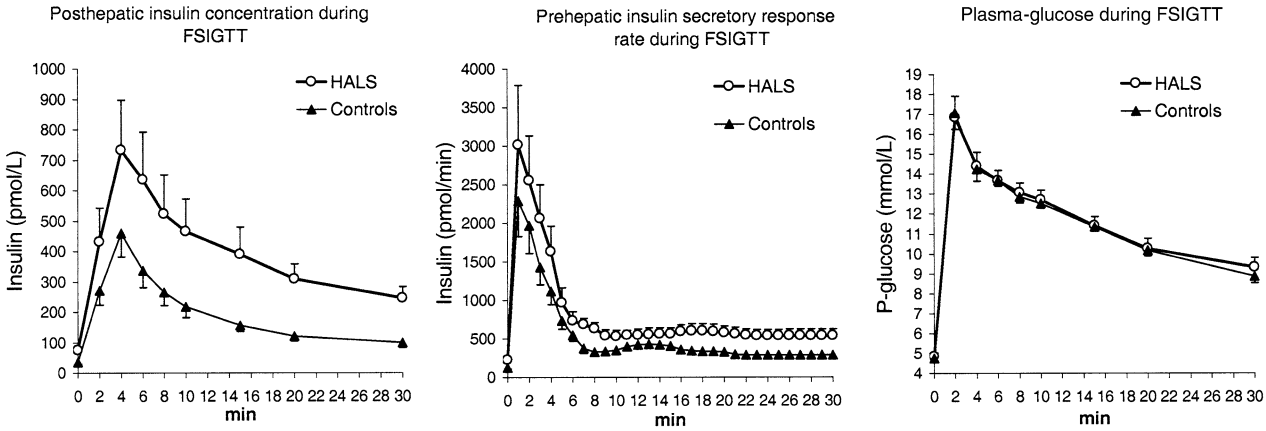

Fig 3. Plots of posthepatic insulin concentration, prehepatic insulin secretory response, and plasma glucose concentration during FSIGTT. During the FSIGTT, no significant difference of $AIRg_{0\text{-}10}$, $AIRs_{0\text{-}6}$, and plasma glucose between HALS patients and control subjects was detected.

respectively). Other independent significant predictors than percentage of limb fat added only little (ie, between 2% to19%) to the explanation of the variations of GDR and NOGM.

β-Cell Function

$AIRg_{0\text{-}10}$ and $AIRs_{0\text{-}6}$ were not statistically different between the 2 groups (Table 1). DI ($Si \cdot AIRg_{0\text{-}10}$) and $Si \cdot AIRs_{0\text{-}6}$ were not statistically different (Table 1). The plots of posthepatic and prehepatic insulin concentration/secretory response and glucose concentration during FSIGTT are illustrated in Fig 3.

The relationship between measures of β-cell function and insulin sensitivity is illustrated in Fig 4A to F. Fasting insulin levels are plotted against Si in Fig 4A. Visual inspection of the data set showed that the relationship between Si and fasting insulin was nonlinear. After a log-log transformation, a linear model fitted the data (Fig 4B), showing a hyperbolic relationship between Si and fasting insulin. The putative hyperbolic relationship between acute incremental insulin response to glucose ($AIRg_{0\text{-}10}$) and Si is illustrated in Fig 4C. The misfit of a hyperbolic relationship between $AIRg_{0\text{-}10}$ and Si might be caused by an insufficient adaptation of β-cell secretion to insulin resistance. Applying the strategy outlined in Materials and Methods, it was found that after omission of the 6 patients with the lowest DI (marked on Fig 4C), no further improvement in the fit of the hyperbolic relationship could be attained. The hyperbolic fit, including the remaining 30 patients (Fig 4D) was then highly significant ($P = .0017$) and significantly improved relative to the fit with all 36 patients ($P = .012$). It appeared that the 6 patients identified in this way were HALS patients, whereas none of the control subjects were identified as having impaired β-cell function ($P < .05$, sign test). Figure 4E shows $AIRs_{0\text{-}6}$ in relationship to Si. Removal of the same 6 HALS patients as above (marked on Fig 4E), showed a significant hyperbolic relationship between $AIRs_{0\text{-}6}$ and Si (Fig 4F), which was significantly improved ($P < .05$) when compared with the fit without removal of the patients.

Comparing these identified 6 HALS patients with presumed impaired β-cell function with the other 12 HALS patients, the former group demonstrated significant impairment, in not only DI, but also $AIRg_{0\text{-}10}$, $AIRs_{0\text{-}6}$, as well as Si (Table 4). Additionally, these 6 HALS patients were characterized by a significantly reduced percentage of limb fat, increased fasting glucose, reduced IV glucose tolerance, and a nonsignificant tendency of increased BMI. In contrast, between groups there was no significant differences in total fat mass, trunk fat mass, peripheral fat mass, WHR, fasting insulin, fasting C-peptide, HOMA-IR, age, free fatty acids, plasma lactate, plasma total cholesterol, HDL cholesterol, and plasma triglycerides. Treatment duration of PI and NRTI was similar in HALS patients with presumed impaired β-cell function and in HALS patients with presumed appropriate β-cell function. In addition, the current antiretroviral treatments were comparable in the 6 HALS patients with relatively inappropriate insulin secretion, in the 12 HALS patients with relatively normal β-cell function, and in the control subjects, respectively (Table 2).

DISCUSSION

The current study showed a 50% reduction of insulin sensitivity in HIV patients who had lipodystrophy (HALS) compared with HIV patients who had not experienced fat redistribution (control subjects). Notably, duration and modality of the antiretroviral treatment were balanced between study groups. Therefore, differences in these parameters may not explain the substantial reduction of insulin sensitivity in lipodystrophic patients. In HALS patients, as in the polled study groups, a strong and independent association between fat distribution and peripheral insulin action appeared, which indicates that lipodystrohy per se may seriously deteriorate insulin sensitivity. Furthermore, in the HALS patients, the NOGM rather than the OGM was impaired. Applying an IV glucose tolerance test to measure acute insulin response, we found that a significant number of the HALS patients compared with control subjects displayed an impaired β-cell function.

The mechanisms of insulin resistance in HALS patients on HAART are not fully understood.[2] Two studies on healthy volunteers taking PI, however, pointed on a direct effect of PI on glucose metabolism.[20,31] This view finds support in an in vitro study, which demonstrated that PI selectively inhibited the activity of the insulin-responsive facilitative glucose trans-

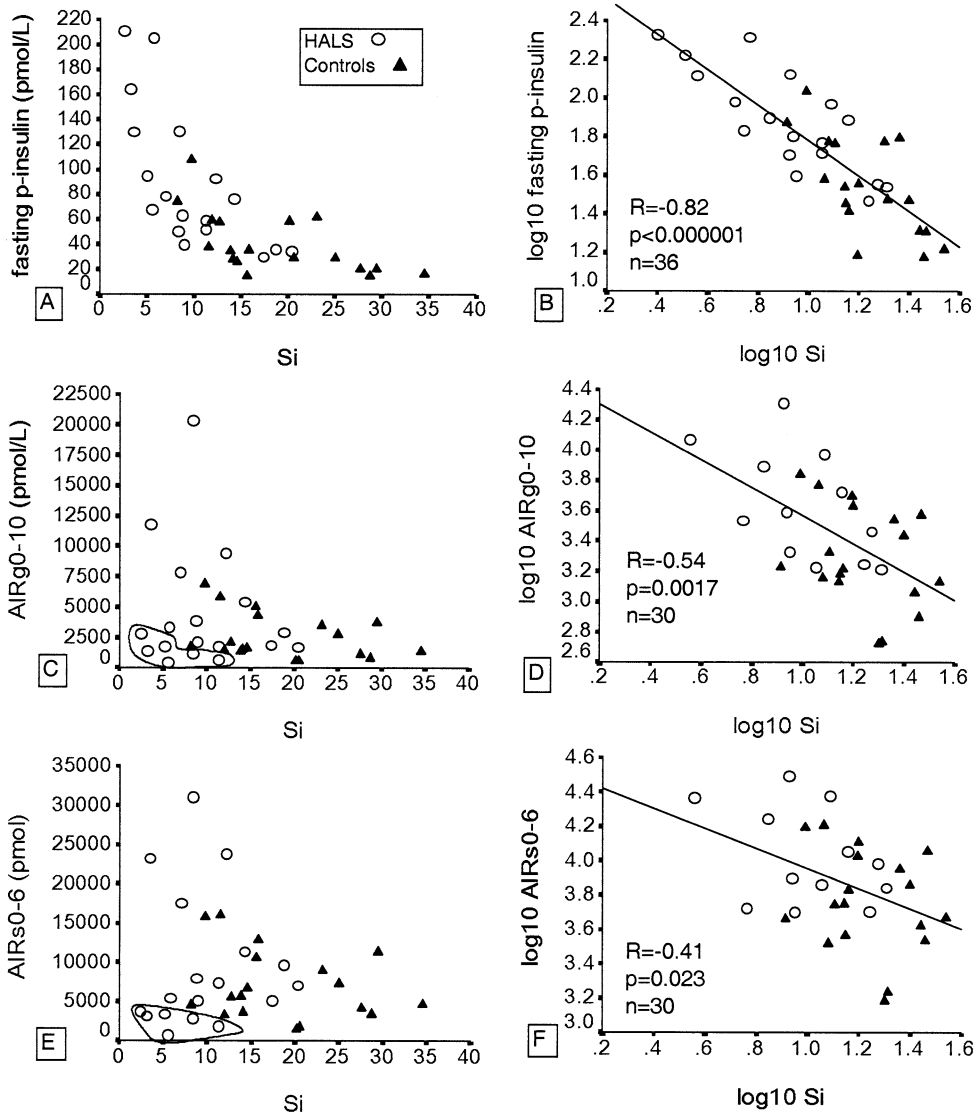

Fig 4. Measures of β-cell function and insulin sensitivity. (A) Plot of fasting insulin levels against Si. Visual inspection of the data set indicated that the relationship between Si and fasting insulin might be hyperbolic. (B) Log-log transformation of the data set in (A). A linear model significantly fitted the data, strongly suggesting a hyperbolic relationship between Si and fasting insulin. (C) Plot of $AIRg_{0\text{-}10}$ and Si. The hyperbolic relationship of these data was not significant ($P = .22$). The 6 patients with the lowest DI (ie, the product of $AIRg_{0\text{-}10}$, and Si) are marked. (D) Log-log transformation of the data set of the unmarked 30 patients in (C). Omission, 1 by 1, of the marked 6 patients with the lowest DI consecutively improved the fit ($P = .012$) resulting in a highly significant hyperbolic fit between $AIRg_{0\text{-}10}$ and Si ($P = .0017$). Omission of additional patient(s) with low DI did not further improve the fit. (E) Plot of $AIRs_{0\text{-}6}$ against Si. The same 6 HALS patients as above are marked. (F) Log-log transformation of the data set of the unmarked 30 patients in (E). A significant hyperbolic relationship was attained between $AIRs_{0\text{-}6}$ and Si ($P = .023$), which was significantly improved ($P < .05$) compared with the fit without omission of patients. The unit of Si is $L^2 \cdot \mu mol^{-1} \cdot min^{-1} \cdot kg^{-1}$.

porter, GLUT-4.[32] In our study, the relatively reduced insulin action in the HALS patients may not, however, be explained as an effect of treatment, because the duration of HAART and the modality of HAART were balanced between study groups. Moreover, we were not able to detect a negative time effect of PI treatment or NRTI treatment on insulin action (Table 3). This observation is in line with the result of a prospective study of Petit et al,[8] who found unchanged fasting glucose and fasting insulin after 12 to 32 months of PI treatment. Recently, in the rat, it has been demonstrated that within a few hours after peak plasma concentration of PI, the direct effect of PI on insulin action vanished.[33] Thus, after 12 hours to18 hours of absence from treatment, a direct effect of antiretroviral treatment on insulin action is likely to be negligible.

The present study design is cross sectional, and the results represent correlations and not cause and effect. It is possible that insulin resistance and hyperinsulinemia occur first and the fat redistribution occurs second in those patients with greater

Table 4. Comparison of HALS Patients With or Without Relatively Appropriate β-Cell Function

	HALS		*P*
	Impaired β Cell	Normal β Cell	
No.	6	12	
DI ($Si \times AIRg_{0\text{-}10}$) (mL/min × kg_{BW})	7 (1)	56 (13)	.003
$AIRg_{0\text{-}10}$ (pmol/L)	1,339 (335)	5,990 (1,617)	0.016
$AIRs_{0\text{-}6}$ (pmol)	2,533 (453)	12,787 (2,550)	.002
Si (L × L/[μmol × min × kg_{BW}])	6.0 (1.3)	11.5 (1.5)	.018
IV glucose tolerance (kg)	3.0 (0.3)	4.1 (0.3)	.031
Percentage of limb fat	33 (1)	38 (2)	.041
BMI (kg/m^2)	26.7 (1.3)	23.8 (0.5)	.09
Lean mass (kg)	64.7 (3.6)	58.5 (2.5)	.19
Total fat mass (kg)	18.4 (2.2)	15.2 (1.9)	.30
Trunk fat mass (kg)	11.7 (1.5)	8.9 (1.2)	.16
Peripheral fat mass (kg)	5.8 (0.7)	5.5 (0.8)	.73
Waist-to-hip ratio	1.03 (0.02)	1.00 (0.02)	.45
Age (yr)	53 (1)	49 (3)	.24
Fasting plasma glucose (mmol/L)	5.5 (0.2)	4.8 (0.2)	.040
Fasting plasma insulin (pmol/L)	108 (27)	80 (15)	.40
Fasting plasma C-peptide (pmol/L)	1,173 (166)	1,132 (178)	.87
HOMA-IR	3.7 (1.0)	2.4 (0.5)	.28
Duration of HIV infection (mo)	103 (28)	97 (17)	.85
Duration of NRTI treatment (mo)	44 (14)	48 (8)	.82
Duration of PI treatment (mo)	26 (5)	35 (5)	.22
Free fatty acids (mmol/L)	0.69 (0.10)	0.60 (0.08)	.47
Lactate (mmol/L)	1.7 (0.2)	1.8 (0.3)	.82
Total plasma cholesterol (mmol/L)	5.9 (0.2)	6.4 (0.6)	.42
Plasma HDL cholesterol (mmol/L)	1.13 (0.10)	0.95 (0.16)	.35
Plasma triglyceride (mmol/L)	2.5 (0.4)	5.3 (1.6)	.11

NOTE. Mean (SEM). IV glucose tolerance during FSIGT was calculated as the slope of the glucose curve between 8 minutes and 30 minutes.

insulin levels, producing the fat redistribution.[34] It could also be speculated that some HIV patients represent a phenotype, which is prone to fat redistribution during HAART, whereas others are relatively resistant to a possible HAART-induced fat redistribution, eg, the control subjects in the present study.

In our HALS patients, the NOGM constituted one half of the level measured in HIV patients without fat redistribution. Previously, in HIV-negative obese patients with a mild type 2 diabetes mellitus, a similar impairment in NOGM has been demonstrated.[35] Reduction in NOGM is associated with impaired activity of glycogen synthase in skeletal muscle.[36] Reduced glycogen synthase might also be one mechanism explaining insulin resistance in HALS, although this remains to be investigated. The basal OGM was similar in both study groups, whereas the insulin-stimulated OGM tended to be reduced in HALS patients compared with control subjects. During the clamp, however, the ratio of OGM to GDR was significantly increased in HALS patients.

Interestingly, in our HALS patients, measures of peripheral fat, but not measures of trunk/abdominal fat, were associated with insulin action, whereas the opposite was found in control subjects (Table 3). Our data thereby provide evidence that the main problem of fat redistribution in HIV patients in relationship to insulin action may be reduction of peripheral fat, rather than trunk fat and abdominal fat accumulation. This is consistent with the findings of Carr et al.[1,7] In their study, however, the HALS patients (n = 87) compared with healthy men were primarily characterized by loss of leg fat and none or only minor concomitant central fat accumulation. They found that the more severe the extremity fat wasting, the more deterioration in insulin sensitivity (calculated from fasting glucose and insulin) and the higher the C-peptide level. In a more recent study, HALS patients exhibited leg fat depletion, which was associated with a high concentration of intramyocellular triglyceride in the soleus muscle of these patients.[37] In agreement with previous findings in HIV negative subjects, the investigators found a negative correlation between amount of skeletal muscle triglyceride and GDR.[38,39] Thus, a mechanism to explain insulin resistance in HALS could be an accumulation of intramyocellular lipid. Speculatively, our finding in HALS patients of a positive correlation between amount of leg fat and NOGM may reflect a higher concentration of muscle triglyceride in those HALS patients with the most pronounced leg fat depletion.

In our study, HALS patients had higher total p-cholesterol than control subjects, whereas no significant differences in free fatty acids, p-HDL cholesterol, and p-triglyceride were detected. Our data are in contrast to the findings from Kosmiski et al,[16] who suggested a relationship between dyslipidemia, insulin action, and fat redistribution in HALS. As an effect of increased general lipolysis due to redistribution of fat tissue[40] and abdominal adiposity per se,[41] an increased flux of free fatty acids to skeletal muscle and the liver may ensue. Thereby, an excess amount of free fatty acids may compete for glucose for oxidation.[42] By consequence, glucose oxidation may decrease and insulin resistance may develop.[43,44] Our data indirectly

support this inference, because our HALS patients compared with the HIV patients without lipodystrophy exhibited a borderline significant increase in free fatty acids and a borderline significant decrease in insulin-stimulated glucose oxidation.

To our knowledge, this is the first study to report data on β-cell function and first phase insulin response in HALS patients evaluated by the use of IV glucose tolerance test. Our data suggest that a significant number of the HALS patients ($n = 6$) compared with HIV patients without lipodystrophy exhibited an insufficient β-cell function in compensation for overt insulin resistance.

The duration and modality of HAART were similar between the 6 HALS patients with relatively impaired insulin secretion compared with the other 12 HALS patients with relatively normal insulin secretion. Interestingly, the former group exhibited decreased percentage of limb fat. These data on insulin secretion suggest that the severity of lipodystrophy, rather than HAART, might be the more important determinant for the impaired insulin secretion. In the fasting condition, all patients seemed to exhibit a β-cell response corresponding to their peripheral insulin sensitivity, as suggested by the strong hyperbolic relationship between fasting insulin level and insulin sensitivity. The general ability in the 6 HALS patients to control fasting normoglycemia (5.5 ± 0.2 mmo/L) indicated a normal β-cell function during fasting. On the other hand, the IV glucose-stimulated insulin secretion in the 6 HALS patients unmasked a β-cell defect.[45,46]

Inadequate first phase insulin secretion to IV glucose bolus injection has been related to an increased risk of developing type 2 diabetes mellitus.[47,48] The 6 HALS patients with relatively impaired insulin secretion and relatively impaired insulin action may therefore be at risk to develop diabetes mellitus, although when the study was undertaken, they exhibited normal fasting plasma glucose.

It is a limitation of this present study that a non-HIV control cohort was not included. However, our aim was to compare HIV patients with fat redistribution with HIV patients without fat redistribution. This enabled us to monitor the isolated effect of lipodystrophy on insulin action in HIV patients. Although the HALS patients had slightly higher BMI, this was due to significantly increased lean mass and not to increased fat mass. The fact that HALS patients were older than control subjects led us to consult the European Group of Insulin Resistance Study (EGIR) who administer a database of hyperinsulinemic euglycemic clamp results from more than 1,000 nondiabetic control subjects who had participated in various clinical trials.[49] It was calculated from the EGIR database that an increase of age from 43 years to 50 years in this background population was not associated with a change in GDR (personal communication, Pernille Poulsen, November 2000).

In conclusion, our data suggest that fat redistribution independently of HAART may play an important role in the pathophysiology of impaired insulin action in male HALS patients compared with male HIV patients without lipodystrophy. Furthermore, the relative impairment in insulin action in HALS patients was applicable to deterioration in the nonoxidative glucose pathway, which indicates a defect in glycogen synthesis in skeletal muscle in this syndrome. Concomitantly to deterioration in insulin sensitivity in male HALS patients, our data support that additional defects in insulin secretion exist.

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