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# In nondiabetic, human immunodeficiency virus–infected patients with lipodystrophy, hepatic insulin extraction and posthepatic insulin clearance rate are decreased in proportion to insulin resistance

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**Abstract**

In healthy, nondiabetic individuals with insulin resistance, fasting insulin is inversely correlated to the posthepatic insulin clearance rate (MCRi) and the hepatic insulin extraction (HEXi). We investigated whether similar early mechanisms to facilitate glucose homeostasis exist in nondiabetic, human immunodeficiency virus (HIV)-infected patients with and without lipodystrophy. We studied 18 HIV-infected patients with lipodystrophy (LIPO) on antiretroviral therapy and 25 HIV-infected patients without lipodystrophy (controls) of whom 18 were on antiretroviral therapy and 7 were not. Posthepatic insulin clearance rate was estimated as the ratio of posthepatic insulin appearance rate to steady-state plasma insulin concentration during a euglycemic hyperinsulinemic clamp (40 $mU \cdot m^{-2} \cdot min^{-1}$). Posthepatic insulin appearance rate during the clamp was calculated, taking into account the remnant endogenous insulin secretion, which was estimated by deconvolution of C-peptide concentrations. Hepatic extraction of insulin was calculated as 1 minus the ratio of fasting posthepatic insulin delivery rate to fasting endogenous insulin secretion rate. Compared with controls, LIPO displayed increased fasting insulin (130%, $P < .001$), impaired insulin sensitivity index (M value , −29%, $P < .001$), and reduced MCRi (−17%, $P < .01$). Hepatic extraction of insulin was similar between groups (LIPO, 55%; controls, 57%; $P > .8$). In LIPO, HEXi and MCRi correlated inversely with fasting insulin ($r = -0.56$, $P < .02$ and $r = -0.68$, $P < .002$) and positively with M value ($r = 0.63$, $P < .01$ and $r = 0.65$, $P < .004$). In controls, MCRi correlated inversely with fasting insulin ($r = -0.47$, $P < .02$) and positively with M value ($r = 0.57$, $P < .004$); however, the correlations between HEXi and these parameters were insignificant ($P > .1$). Our data suggest that HEXi and MCRi are decreased in proportion to the degree of insulin resistance in nondiabetic HIV-infected patients with lipodystrophy.


## 1. Introduction

To compensate for an attenuating insulin sensitivity, nondiabetic individuals increase their insulin secretion [1]. However, the liver extracts approximately 50% to 60% of the insulin secreted from the pancreas during the first portal passage [2,3]. Regulation of the hepatic clearance of insulin, therefore, plays a role in the concentration of plasma insulin. It has been demonstrated that nondiabetic individuals reduce hepatic extraction of insulin (HEXi) as insulin resistance increases, and higher concentrations of plasma insulin are needed [4-6]. In addition, the systemic clearance of insulin has been shown to be reduced in nondiabetic individuals with obesity [7] and in lean individuals with insulin resistance [8].

During recent years, lipodystrophy in human immunodeficiency virus (HIV)–infected patients on combined highly active antiretroviral therapy (HAART) has been

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increasingly commonly described [9-13]. The prevalence of this syndrome in HIV-infected patients on HAART approximates 40% [14]. A mixed lipodystrophy (ie, peripheral fat atrophy and central fat accumulation with a normal body mass index [BMI] and without an increased total fat mass) is the most frequent type [15]. Insulin resistance is closely related to HIV lipodystrophy, although frank diabetes mellitus is rare, and patients often present a normal fasting glucose level [11-13,16]. However, increased fasting insulin and C-peptide levels in HIV-infected patients with lipodystrophy reflect a compensated state of insulin resistance [12,15,16]. We hypothesize that insulin-resistant, nondiabetic, HIV-infected patients with lipodystrophy, besides increased prehepatic insulin secretion, might show reduced hepatic extraction and systemic clearance of insulin to facilitate glucose homeostasis.

We studied normoglycemic, nondiabetic, HIV-infected patients with and without lipodystrophy using data from a euglycemic hyperinsulinemic clamp to estimate insulin sensitivity. Measurements of plasma insulin and C-peptide during fasting and clamp steady-state period enabled the assessment of prehepatic insulin secretion, posthepatic insulin clearance rate (MCRi), and HEXi, combining previously validated methods [3,17-19].

## 2. Methods

### 2.1. Study subjects

Forty-three HIV-1-positive men were recruited from the outpatient clinic of infectious diseases at Hvidovre Hospital, University of Copenhagen, Denmark. Out of these, 36 HIV-infected patients were receiving HAART, including 18 who displayed lipodystrophy (LIPO) and 18 who were without sign and without complaints related to lipodystrophy (NONLIPO). Seven HIV-infected patients were naive to HAART (NAIVE) and had no sign and no complaint of lipodystrophy. Inclusion and exclusion criteria including anthropometric and glucose metabolic data for the HIV-infected patients on HAART have previously been described in detail [20]. In short, for LIPO a patient-administered questionnaire must be positive for at least one criterion of lipodystrophy (ie, for lipoatrophy: loss of fat in the face, arms, legs, buttocks, more exposed veins; and for lipoaccumulation: gain of fat at abdomen/trunk, fat pads in the neck region). Physical examination result must be positive for at least 1 sign of lipodystrophy (examination for lipoatrophy in the face, extremities, and buttocks; and for lipoaccumulation, in abdomen/trunk and buffalo hump). For the control subjects (ie, NONLIPO and NAÏVE), both questionnaire and physical examination must be negative. All LIPO, except one who had multiple lipomatosis, displayed lipodystrophy consistent with peripheral fat atrophy and central fat accumulation (ie, mixed lipodystrophy). Except for HAART, none of the subjects received medication known to affect glucose metabolism. All participants, but 2 NONLIPO, had negative family history of diabetes mellitus, and all participants, but 2 NONLIPO, were of Caucasian 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. Study protocol

Instructions were given to abstain from strenuous exercise for at least 3 days before the metabolic assessments. The HIV-infected patients reported to our laboratory at 8:00 AM after a 12-hour overnight fast including at least 16 hours of abstinence from HAART. The study protocol has been described previously [20]. At 8:30 AM, 2 intravenous cannulas were inserted, one in a dorsal hand vein and the other in an antecubital vein. The hand vein was used for blood sampling, whereas the antecubital vein was used for infusion. After a 150-minute basal period (0-150 minutes), a 30-minute intravenous glucose tolerance test (IVGTT, 150–180 minutes) and a 120-minute hyperinsulinemic euglycemic clamp (180–300 minutes) were conducted. Two steady-state periods were predefined (ie, at 90–120 minutes as basal and 270-300 minutes as clamp). The 30-minute IVGTT was initiated with a 1-minute glucose infusion (0.3 g/kg body weight). Thereafter, insulin (Actrapid, Novo Nordisk A/S, Bagsvaerd, Denmark) infusion was started with a stepwise decline in infusion rate every third minute from 100 to 80 to 60 to 40 $mU \cdot m^{-2} \cdot min^{-1}$; hereafter (+9 to 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, blood samples were taken every 5 minutes for measurement of glucose, and blood samples for assessing plasma insulin and C-peptide levels were taken every 15 minutes during the first 90 minutes of the 120-minute clamp period and every 10 minutes during predefined steady-state period (ie, last 30 minutes of the clamp). Body composition was evaluated by dual-energy x-ray absorptiometry (Norland Medical System XR-36, Fort Atkinson, WI). To estimate the amount of fat in the trunk (chest, abdomen, and pelvis) and in the extremities, a whole-body scan was performed. The proximal limitation of the leg region was placed through the hip joints at an angle of approximately 45°. The dual-energy x-ray absorptiometry scans were done in random order, and the operator was unaware of the assignment of the patients to study groups.

### 2.3. Assays

Whole-blood glucose concentrations were determined pairwise on two calibrated HemoCue B glucose analyzers (HemoCue 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 and D'Orazio [21]

from whole-blood glucose (the mean of the pair of measurements) and hematocrit. Blood samples for plasma insulin and C-peptide were centrifuged immediately at 4°C and stored at −80°C for later analysis 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, 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 to 33 split proinsulin, and 92% with 64 to 65 split proinsulin; there was no detectable cross-reactivity with insulin, intra-assay CV of 5%, and interassay CV of 8%. Plasma free fatty acids (FFAs) were determined using an enzymatic colorimetric method (Wako C test kit, Wako Chemicals GmbH, Neuss, Germany) with an interassay CV of 5%. Total plasma cholesterol and triglycerides were determined by reflection photometry (Ortho-Clinical Diagnostics kit, Raritan, NJ) with interassay CV of 2% and 2.5%. CD4 count determination (flow cytometry, Becton-Dickinson FACscan, BD, Franklin Lakes, NJ; interassay CV of 7%) and viral load determination (Roche Amplicor and amplicer ultrasensitive assay with a detection limit of 20 copies per milliliter of plasma, Roche, Basel, Switzerland) met the requirements of interlaboratory quality control.

### 2.4. Calculations

The mean glucose infusion rate during the predefined steady-state period of the clamp (ie, at 270-300 minutes) was expressed per kilogram fat-free body mass and defined as the insulin sensitivity index (M value, in $\mu$mol glucose $\cdot\, kg_{FFM}^{-1} \cdot min^{-1}$).

Prehepatic insulin secretion rates (ISRs) were calculated from plasma C-peptide measurements using the ISEC (Insulin SECretion) computer program [17]. The method is based on assumptions that insulin and C-peptide are cosecreted in an equimolar fashion by the pancreas [22] and that C-peptide is not cleared by the liver and its kinetics is linear over a wide range of plasma concentrations [23]. ISEC does not use plasma insulin concentration. ISEC has been validated to calculate ISR during IVGTT [24] and has been applied to calculate prehepatic insulin secretion profiles during meal tolerance test [25], hyperinsulinemic euglycemic clamp, and during basal conditions [17].

The ratio of the basal posthepatic insulin delivery rate ($IDR_{basal}$) (ie, the rate at which endogenous insulin is delivered to the systemic circulation after transhepatic passage) to basal ISR is, by definition, the fraction of insulin not extracted by the liver [3]. Thus, the fractional HEXi was calculated from the following equation [3]:

$$HEXi = 1 - (IDR_{basal} \;/\; ISR_{basal}) \quad (1)$$

During the basal period, plasma insulin levels are in steady state; thus, $IDR_{basal}$ can be calculated as the product of basal plasma insulin concentration and the MCRi [3]:

$$IDR_{basal} = p - insulin_{basal} \times MCRi \quad (2)$$

Posthepatic insulin clearance rate was estimated as the ratio of the total appearance rate of insulin in the systemic circulation during the clamp steady-state period ($TARI_{clamp}$) to plasma insulin concentration during clamp steady-state period ($p\text{-}insulin_{clamp}$) as suggested previously [18]:

$$MCRi = TARI_{clamp} \;/\; p - insulin_{clamp} \quad (3)$$

The assumption was made that the insulin clearance determined during the clamp with insulin concentration of 300 to 800 pmol/L also applies to the basal range of plasma insulin concentrations of 20 to 200 pmol/L. The validity of this assumption has been verified in studies using the euglycemic insulin clamp technique, in which reduced liver extraction of insulin was seen only at insulin concentrations greater than 1200 pmol/L, and peripheral clearance was shown to be constant, at least up to an insulin concentration of 600 pmol/L [2,26].

$TARI_{clamp}$ was calculated as the sum of exogenous infusion rate of insulin (IIR) and the endogenous insulin delivery rate to the systemic circulation during the clamp ($IDR_{clamp}$). The latter was calculated as ISR during clamp ($ISR_{clamp}$) minus the assumed HEXi, which initially was chosen to be 50%. As it turned out, the mean HEXi was close to 55%; this percentage was chosen in the final calculation. Thus, the following equation was calculated:

$$TARI_{clamp} = IIR + \left(ISR_{clamp} \times 0.45\right) \quad (4)$$

It should be noted that an error of the initial estimate of HEXi of, for example, 10% results only in 1% to 2% error in the final calculation of HEXi because the exogenous insulin infusion rate is much higher than $IDR_{clamp}$. In the present study, $IDR_{clamp}$ approximated to 20% of IIR in LIPO and 10% in NONLIPO and NAÏVE.

### 2.5. Statistics

Data are presented as means ± SEM and as medians and ranges when distributions were skewed. Student $t$ test was used to compare the means between cases and control subjects. Analysis of variance (ANOVA) was applied to compare the means of the 3 clinical groups. Pearson correlation coefficient ($r$) was applied for assessment of linear correlation between variables. Adjustment for age and percentage of total fat mass was performed by calculating the partial correlation coefficient between variables. If data distributions were skewed, a log transformation was made before applying $t$ test and calculation of a correlation coefficient. Calculations were performed by SPSS version 10.0.7 (SPSS Inc, Chicago, IL). Two-sided $P$ values larger than .05 were regarded as statistically not significant.

## 3. Results

### 3.1. Characteristics of study groups

LIPO patients were older than NONLIPO and NAÏVE patients (50 ± 2 vs 43 ± 2 vs 36 ± 3 years, all *P*s < .05). Although LIPO exhibited increased BMI compared with NONLIPO (24.7 ± 0.6 vs 22.5 ± 0.8 kg/m$^2$, $P = .04$), this was not accompanied by a significant increase in fat mass (16 ± 1 vs 13 ± 2 kg, $P = .13$), while there was an increased lean body mass (61 ± 2 vs 54 ± 2 kg, $P = .03$) in LIPO. Body mass index was similar in LIPO and NAÏVE (24.8 ± 1.0 kg/m$^2$). Total fat mass in NAÏVE was 20 ± 2 kg, which was increased compared with NONLIPO ($P < .05$) but similar to LIPO ($P > .2$). Trunk fat mass was increased in LIPO compared with NONLIPO (10 ± 1 vs 7 ± 1 kg, $P = .02$) but similar to NAÏVE (10 ± 1 kg). Ratio of limb fat to trunk fat mass was significantly reduced in LIPO (58 ± 4%) compared with NONLIPO (89 ± 7%, $P < .001$) and NAÏVE (92 ± 5%, $P < .001$), consistent with mixed lipodystrophy in LIPO.

Duration of HIV infection was not significantly different between the 3 groups (LIPO, 99 ± 14 months; NONLIPO, 72 ± 11; and NAÏVE 61 ± 25; all *P*s > .14). All LIPO and all NONLIPO were treated with nucleoside reverse transcriptase inhibitors as part of HAART, and mean duration of therapy for this class of drug was 47 ± 7 and 42 ± 6 months, $P = .6$. Frequently used nucleoside reverse transcriptase inhibitors in LIPO and NONLIPO were lamivudine (83%), zidovudine (50%), and stavudine (44%), respectively. As part of HAART, protease inhibitors were used by 90% of LIPO and NONLIPO. Frequently used protease inhibitors were indinavir (33%), ritonavir (31%), and nelfinavir (22%). Components of HAART were balanced between the two groups. CD4 cell numbers were similar between study groups (LIPO, 427 ± 45 cells per microliter; NONLIPO, 352 ± 46; and NAÏVE, 521 ± 90; all *P*s > .05), whereas LIPO and NONLIPO displayed lower numbers of HIV particles than NAÏVE (median, < 20 vs 14 900 copies per milliliter; $P < .05$).

Basal plasma FFA concentration did not differ significantly between study groups (LIPO, 0.57 ± 0.05 mmol/L; NONLIPO, 0.54 ± 0.04; NAÏVE, 0.44 ± 0.07 mmol/L; all *P*s > .1). Basal plasma triglyceride was increased in LIPO (median, 2.6 mmol/L; range, 0.8-16.0) compared with NONLIPO (median, 1.8 mmol/L; range, 0.6–5.4; $P = .1$) and NAÏVE (median, 1.7 mmol/L; range, 1.0–3.6; $P = .05$). Total plasma cholesterol was increased in LIPO (6.2 ± 0.4 mmol/L) compared with NONLIPO (4.9 ± 0.2, $P < .01$) and NAÏVE (4.7 ± 0.4, $P < .05$).

### 3.2. MCRi and HEXi including other glucose metabolic parameters

Compared with NONLIPO and NAIVE, LIPO displayed an impaired M value (−33%, $P < .001$, and −18%, $P = .07$) (Table 1). Posthepatic insulin clearance rate was significantly reduced in LIPO relatively to NONLIPO (−21%, $P < .002$) but similar relatively to NAÏVE ($P > .5$). Hepatic extraction of insulin was similar between groups (LIPO, 55%; NONLIPO, 57%; NAÏVE, 57%; ANOVA, $P > .9$).

Mean basal glucose concentrations were similar in the groups (range, 4.80-4.85 mmol/L; $P = .7$), and all subjects had basal plasma glucose of 6.1 mmol/L or less. Mean clamp glucose concentrations were similar between LIPO (5.08 ± 0.04 mmol/L), NONLIPO (5.11 ± 0.11 mmol/L), and NAÏVE (5.09 ± 0.06 mmol/L). Peak and mean plasma glucose concentrations during the IVGTT were not different between study groups ($P > .3$). Basal and clamp insulin concentrations were increased in LIPO (77 ± 11 and 535 ± 35 pmol/L) compared with NONLIPO (32 ± 4 and 392 ± 16 pmol/L; all *P*s < .01) and NAÏVE (37 ± 5 and 445 ± 26 pmol/L; all *P*s < .01). Also, basal and clamp C-peptide concentrations were increased in LIPO (1145 ± 128 and 1442 ± 232 pmol/L) compared with NONLIPO (650 ± 75 and 610 ± 59 pmol/L, $P < .01$) and NAÏVE (525 ± 59 and 674 ± 99 pmol/L, $P < .01$).

Compared with NONLIPO and NAÏVE, LIPO displayed increased fasting ISR (68% and 71%, $P < .005$) and increased fasting IDR (77% and 81%, $P < .003$) (Table 1). Clamp ISR did not differ significantly from basal ISR in LIPO (3.4 ± 0.5 vs 2.7 ± 0.3 pmol · kg$^{-1}$ · min$^{-1}$, $P > .1$), NONLIPO (1.6 ± 0.2 vs 1.6 ± 0.2 pmol · kg$^{-1}$ · min$^{-1}$, $P > .9$), and NAÏVE (1.6 ± 0.2 vs 1.6 ± 0.3 pmol · kg$^{-1}$ · min$^{-1}$, $P > .9$),

Table 1
Data on insulin action, beta-cell function, insulin clearance, and HEXi

| | LIPO | NONLIPO | NAÏVE | *P* | | | ANOVA (all groups) |
|---|---|---|---|---|---|---|---|
| | | | | L vs NL | L vs NA | NL vs NA | |
| Number and gender | 18 males | 18 males | 7 males | | | | |
| Fasting plasma insulin (pmol/L) | 77 (11) | 32 (4) | 37 (5) | .002 | .004 | NS | <.001 |
| M value ($\mu$mol · kg$_{FFM}^{-1}$ · min$^{-1}$) | 31 (3) | 46 (3) | 38 (2) | <.001 | .07 | .03 | <.002 |
| MCRi (mL · kg$^{-1}$ · min$^{-1}$) | 17.4 (1.0) | 22.2 (0.9) | 18.2 (0.8) | .002 | NS | .006 | <.003 |
| ISR$_{BASAL}$ (pmol · kg$^{-1}$ · min$^{-1}$) | 2.7 (0.3) | 1.6 (0.2) | 1.6 (0.2) | .002 | .004 | NS | <.002 |
| IDR$_{BASAL}$ (pmol · kg$^{-1}$ · min$^{-1}$) | 1.20 (0.13) | 0.69 (0.07) | 0.66 (0.08) | .002 | .008 | NS | <.002 |
| HEXi (%) | 55 (3) | 57 (3) | 57 (3) | NS | NS | NS | NS |

Data are means (SEM).
L and NL indicate LIPO and NONLIPO, respectively (ie, HIV-infected patients with and without lipodystrophy, respectively, who had been on therapy with antiretroviral drugs for more than 12 months); NA, NAÏVE (NONLIPO naive to antiretroviral drugs). M value is calculated as glucose infusion rate divided by kilograms fat-free body mass.

Table 2
Correlation coefficients between HEXi and MCRi vs fasting plasma insulin and insulin sensitivity

| | Total (n = 43) | | LIPO (n = 18) | | Controls (n = 25) | |
|---|---|---|---|---|---|---|
| | FP insulin | M value | FP insulin | M value | FP insulin | M value |
| HEXi | −0.39** (−0.32*) | 0.24 (0.14) | −0.56* (−0.51*) | 0.63** (0.48) | −0.33 (−0.27) | −0.03 (−0.24) |
| MCRi | −0.65*** (−0.60***) | 0.68*** (0.61***) | −0.68** (−0.64**) | 0.65** (0.59*) | −0.47* (−0.19) | 0.57** (0.37) |

Pearson correlation coefficients, *$P < .05$, **$P < .01$, ***$P < .001$. Values in parentheses are partial correlation coefficients adjusted for age and percentage of total fat mass. Controls indicate the combined group of NONLIPO and NAÏVE.
FP insulin indicates fasting plasma insulin; M value, insulin sensitivity index derived from the clamp study.

respectively. The missed suppression of ISR during clamp might be explained by the preceding IVGTT inducing a second-phase insulin response, which is likely superimposed on the clamp steady-state period. Plasma insulin concentration during the last 60 minutes of the 120-minute clamp did not differ significantly from the insulin concentration during the predefined steady-state period (ie, the last 30 minutes of the 120-minute clamp). Likewise, glucose concentration and ISR during the last 75 minutes of the 120-minute clamp were not significantly different from glucose concentration and ISR at the predefined steady-state period of the clamp.

### *3.3. Correlations between HEXi and MCRi vs fasting insulin, M value, and parameters of fat mass and fat distribution*

In the total group, HEXi correlated inversely with fasting insulin ($r = −0.39$, $P < .01$, n = 43) but not with M value ($r = 0.24$, $P = .11$, n = 43) (Table 2 and Fig. 1). However, removal of the single outlier as seen in Fig. 1 (a NONLIPO patient) improved the correlation between HEXi and fasting insulin in the total group ($r = −0.48$, $P < .002$, n = 42) and made the correlation between HEXi and M value highly significant ($r = 0.49$, $P = .001$, n = 42). In LIPO, HEXi correlated with fasting insulin ($r = −0.56$, $P < .02$) and M value ($r = 0.63$, $P < .01$). In the combined group of NONLIPO and NAÏVE (ie, control subjects), such correlations failed to reach significance (Table 2) even after the removal of the outlier (HEXi vs insulin: $r = −0.32$, $P = .13$; and HEXi vs M value: $r = 0.30$, $P = .16$). Posthepatic insulin clearance rate correlated inversely with fasting insulin and positively with M value in the total group ($r = −0.65$, $P < .001$ and $r = 0.76$, $P < .001$), in LIPO ($r = −0.68$, $P < .002$ and $r = 0.65$, $P < .004$), and in control subjects ($r = −0.47$, $P < .02$ and $r = 0.57$, $P < .004$) (Table 2 and Fig. 2).

Because age differed between groups and has been shown to play a role in at least MCRi in HIV-negative individuals, albeit of minor importance, when considering the range of age for the participants in the present study [18], we adjusted for age in the correlations presented above. Also, obesity has been shown to relate to MCRi; therefore, correlations were adjusted for percentage of total fat mass [7]. It appeared that the correlations mentioned above in the LIPO group, but not in control subjects, were robust for adjustment for age and percentage of total fat mass (Table 2). This was partially explained as total fat mass and trunk fat mass, but not ratio of limb fat, correlated significantly with MCRi in control subjects ($r = −0.53$, $P < .01$; $r = −0.43$, $P < .05$; $r = 0.28$, $P > .15$), whereas in LIPO the correlations of MCRi to total fat mass ($r = −0.29$, $P > .2$) and trunk fat mass ($r = −0.38$, $P > .1$) were insignificant; however, a significant correlation was detected between MCRi and ratio of limb fat ($r = 0.48$, $P < .05$) in LIPO. Correlations between HEXi and these measures of fat

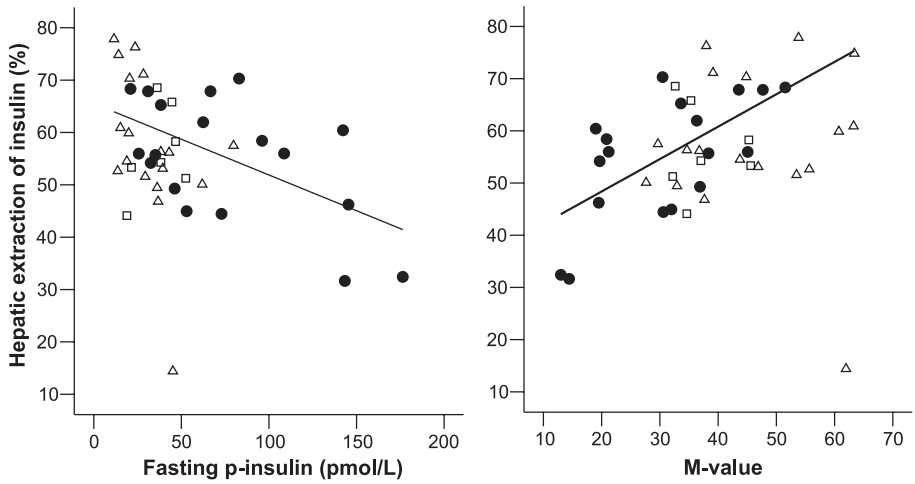


Fig. 1. Scatterplots of the correlations of the HEXi vs fasting plasma insulin and M value. Filled circles indicate LIPO patients; open triangles, NONLIPO; open rectangles, NAÏVE; and solid lines, linear correlations (LIPO). Correlation coefficients and $P$ values are given in Table 2.

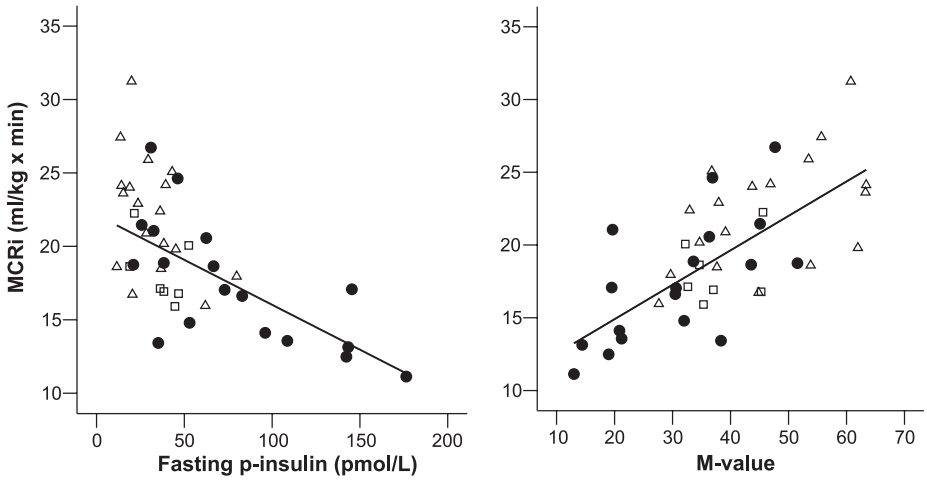


Fig. 2. Scatterplots of the correlations of the MCRi vs fasting plasma insulin and M value. Filled circles indicate LIPO patients; open triangles, NONLIPO; open rectangles, NAÏVE; and solid lines, linear correlations (LIPO). Correlation coefficients and *P* values are given in Table 2.

mass and distribution did not reach statistical significance in any of the study groups. Basal plasma concentrations of FFA and triglyceride did not correlate significantly with MCRi and HEXi in any of the study groups.

## 4. Discussion

Our main objective was to investigate whether nondiabetic, insulin-resistant, HIV-infected patients with lipodystrophy down-regulate clearance of insulin, thus alleviating the beta cell for the increasing demand for insulin secretion that follows impaired insulin sensitivity [27]. In the group of HIV-infected patients with lipodystrophy, inverse correlations were found between fasting plasma insulin and insulin sensitivity vs measures of hepatic and systemic insulin clearance. Accordingly, our data suggest that insulin-resistant HIV-infected patients with lipodystrophy diminish overall insulin clearance to facilitate the demand for higher concentrations of insulin required by insulin-resistant tissues.

To our knowledge, this is the first study to address HEXi in HIV-infected patients. It has been demonstrated that in addition to insulin hypersecretion in obese humans with insulin resistance, HEXi is reduced [3,4,6,28]. The extraction of insulin during the first pass in the liver approximates a mean value of 50% to 60%, which is consistent with our present findings in HIV-positive men [2,3,26]. Recent studies indicate that FFAs within physiological range play no role or just a minor role in the HEXi in nondiabetic humans and dogs [29,30], which is consistent with our results (ie, we did not find a significant correlation between levels of lipidemia and HEXi). Incretins have been shown to lower HEXi [31,32] and could therefore be a mechanism by which nondiabetic, insulin-resistant, HIV-infected patients down-regulate HEXi. In the present study, we did not measure incretins, but this issue clearly merits research. In addition, insulin per se might down-regulate insulin receptor numbers in the liver and thereby impair the HEXi process [33], which corresponds with the inverse correlations between HEXi and plasma insulin in the total study group and in the LIPO group in the present study. Increased hepatic triglyceride content has been shown to correlate inversely with insulin clearance in HIV-negative individuals [34,35], so it might be speculated that a similar mechanism operates in HIV-infected patients with lipodystrophy. In this context, it is of interest that increased amounts of triglyceride in the liver (measured using proton spectroscopy) of HIV-infected patients with lipodystrophy correlated with increased fasting plasma levels of insulin and C-peptide [36].

The nonsignificant correlations between HEXi, fasting insulin, and M value in the combined group of NONLIPO and NAÏVE (ie, control subjects, n = 25) most likely signify that these control subjects had lower fasting insulin and higher M value and displayed a more narrow span of these parameters compared with the HIV-infected patients with lipodystrophy. In the total group, after the removal of the outlier with very low HEXi (Fig. 1), the correlations between HEXi, plasma insulin, and M value were highly significant, probably reflecting that nondiabetic HIV-infected patients regulate HEXi in proportion to insulin concentration and insulin sensitivity.

A previous study has shown reduced systemic insulin clearance in 6 insulin-resistant HIV-infected patients with lipodystrophy who were on HAART compared with 6 insulin-sensitive, therapy-naive, HIV-infected patients [37]. In another study, systemic insulin clearance was reduced in former protease inhibitor–naive HIV-infected patients after 12 weeks of therapy with protease inhibitors [38]. Thus, an effect of protease inhibitors on the level of systemic clearance of insulin in HIV-infected patients might be expected. Our study did not address this aspect. In fact, components of HAART and duration of HAART were balanced between LIPO and NONLIPO. Furthermore, it should be considered that all patients on HAART in the present study were abstinent from therapy at least 16 hours before the start of experiments. It has been shown that the negative impact of a protease inhibitor upon overall

glucose metabolism is likely to be small within a few hours after abstinence from the drug, albeit this was demonstrated in a rat model [39]. Therefore, it is likely that a direct effect of the component of HAART on insulin clearance would be small in the present study and that other factors related to the role of fat redistribution may play a more important role.

In theory, diminished HEXi and reduced posthepatic clearance of insulin could be the primary events leading to hyperinsulinemia, in turn causing the insulin resistance as minute increments in fasting insulin concentrations may induce insulin resistance [40]. Alternatively, one may hypothesize that a down-regulation of the overall insulin receptor function in HIV-lipodystrophy as has been detected in obesity [41] may lead to reduced receptor-dependent insulin degradation, explaining the reduced systemic insulin clearance in HIV-infected patients with lipodystrophy. However, the present study is cross-sectional and cannot determine whether decreased hepatic extraction and posthepatic clearance of insulin are an effect of other factors or a primary factor. A prospective study would be needed to clarify the nature of regulation of these factors in HIV-infected patients with lipodystrophy.

The method to calculate HEXi in the present study [3] allows one to obtain an absolute value of this parameter and thereby is superior to the mere ratio of fasting insulin to fasting C-peptide, which has been implemented previously as an index for HEXi [4]. However, other methods than the present model may provide similar precision for the estimation of an absolute value of HEXi, for example, the frequently sampled intravenous glucose tolerance test computed to the minimal model [42,43], because both methods rely on population-based studies of C-peptide kinetics [19,44]. Direct measurement of transhepatic insulin clearance rate requires catheterization and is very laborious and inconvenient for the patient; however, such studies have been performed in humans [2] and dogs [45], showing levels of HEXi of 40% to 60%, which matches our present finding.

The insulin infusion rate used in the present study (40 mU $\cdot$ $m^{-2}$ $\cdot$ $min^{-1}$) has been shown to suppress hepatic glucose production by more than 75% [46,47]; in addition, the remnant hepatic glucose production at this insulin infusion rate is likely similar between individuals who display a broad range of insulin sensitivity [47]. Furthermore, hepatic glucose production has been shown to constitute less than 15% of the rate of peripheral glucose disposal during such clamp settings even in patients with type 2 diabetes mellitus [47]. Nevertheless, we cannot completely exclude the possibility that the relationship between decreased HEXi and peripheral glucose disposal (ie, exogenous glucose infusion, M value) in the present study may be due to a failure of insulin to suppress hepatic glucose production (thus lowering the need for exogenous glucose infusion and M value) in individuals with decreased HEXi. This would still be consistent, however, with the conclusion drawn from the present results that HEXi is decreased in proportion to insulin resistance, but the insulin resistance would be more at the level of the liver than peripherally.

The present study did not include HIV-negative controls, and therefore we must rely on historical data to evaluate whether the levels of HEXi and systemic insulin clearance in HIV-infected patients are within reference range. In a large cohort of healthy Caucasian men, mean MCRis of 45 to 50 mL $\cdot$ $m^2$ $\cdot$ $min^{-1}$ $\cdot$ $kg^{-1}$ (ie, whole-body MCRi divided by BMI) were estimated using hyperinsulinemic (1 mU $\cdot$ $kg^{-1}$ $\cdot$ $min^{-1}$) euglycemic clamps [18]. However, these investigators did not correct for the endogenous insulin secretion, which probably is suppressed by approximately 50% in this setting [48,49]. The average fasting plasma insulin concentration was 60 to 80 pmol/L in these HIV-negative individuals [18]. Given the calculated systemic insulin clearance rate and a HEXi of approximately 50% and a clamp insulin concentration of 400 to 500 pmol/L, the posthepatic delivery rate of insulin during the clamp would be underestimated by approximately 10% in these HIV-negative individuals. Correcting for this systematic error, the "normal" mean MCRi may approximate 50 to 55 mL $\cdot$ $m^2$ $\cdot$ $min^{-1}$ $\cdot$ $kg^{-1}$, which is very close to the mean posthepatic clearance rate in our HIV-infected patients with lipodystrophy of 55 mL $\cdot$ $m^2$ $\cdot$ $min^{-1}$ $\cdot$ $kg^{-1}$, who had similar fasting plasma insulin concentrations. The HIV-infected control subjects without lipodystrophy in the present study exhibited a slightly higher MCRi of 65 mL $\cdot$ $m^2$ $\cdot$ $min^{-1}$ $\cdot$ $kg^{-1}$ but had lower fasting insulin than these historical controls, which probably explains the difference, given the inverse correlation between systemic insulin clearance and plasma insulin concentration [18].

In conclusion, our data suggest that nondiabetic, insulin-resistant, HIV-infected patients with lipodystrophy regulate HEXi and systemic clearance of insulin in proportion to plasma insulin concentration and insulin resistance, which may be compensatory mechanisms to facilitate glucose homeostasis in insulin-resistant tissues of such individuals.

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