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Impaired proinsulin secretion before and during oral glucose stimulation in HIV-infected patients who display fat redistribution

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Abstract

The beta-cell function of HIV-infected patients on highly active antiretroviral therapy who display lipodystrophy may be impaired. An early defect in beta-cell function may be characterized by an increase in secretion of 32-33 split proinsulin (SP) and intact proinsulin (IP). To address this issue, the secretion patterns of SP and IP of 16 HIV-infected men with lipodystrophy (LIPO) and 15 HIV-infected men without lipodystrophy (NONLIPO) were studied during an oral glucose tolerance test (OGTT). All patients received highly active antiretroviral therapy. Insulin secretion rates were determined by deconvolution of plasma C-peptide concentrations. More LIPO than NONLIPO patients displayed diabetes mellitus and impaired glucose tolerance than normal glucose tolerance (LIPO 2/8/6 vs NONLIPO 1/2/12, $P = .05$). LIPO patients had increased fasting levels of SP and IP, ratio of SP/IP, and area under the curve of SP and IP during the early phase (0, 10, and 20 minutes) and during the late phase (45, 75, and 105 minutes) of the OGTT compared with NONLIPO patients (Ps $< .05$). LIPO patients exhibited significantly increased fasting SP/IP ratio, fasting SP/insulin ratio, and total proinsulin to C-peptide ratio during the OGTT. LIPO patients displayed increased incremental secretion of IP during the first 10 minutes of the OGTT ($P < .05$), although the incremental insulin secretion during this period did not differ between LIPO and NONLIPO patients. These data suggest that HIV-infected patients with lipodystrophy display major perturbations of proinsulin secretion in the fasting state and during an OGTT, which is compatible with the notion of a beta-cell dysfunction of such patients.

1. Introduction

It is a well-established fact that HIV-infected patients with lipodystrophy exhibit a cluster of metabolic aberrations, eg, insulin resistance [1-3], impaired glucose tolerance [2,4,5], hyperlactatemia [6,7], dyslipidemia [1,8], and increased levels of circulating tumor necrosis factor α and other proinflammatory cytokines [9,10]. The mechanisms of this dysmetabolic phenotype are multifactorial and are likely to be associated with the antiretroviral therapy, genetic susceptibility of the individual, lifestyle, the HIV infection itself, and, in some cases, coinfection with hepatitis [11,12]. The dysmetabolism of HIV-infected patients on highly active antiretroviral therapy (HAART) may significantly increase risk of cardiovascular disease (CVD) [13]. The risk of CVD and specifically myocardial infarction in HIV-infected patients may increase per year under HAART [14].

Few articles have addressed beta-cell function in HIV-infected patients undergoing HAART [5,15-17]. Hyperinsulinemia due to impaired beta-cell function may cause insulin resistance [18]. Beta-cell dysfunction and insulin resistance together cause type 2 diabetes mellitus (T2DM) [19], impaired lipid metabolism [20], and, likely, increased release of tumor necrosis factor α [21]. A healthy beta-cell function includes a normal insulin-processing machinery, which is characterized by an appropriate secretion of proinsulin [22,23]. Therefore, in vivo, the ratio of proinsulin to insulin has been used as an index of proinsulin processing. In addition, in an HIV-negative population, increased secretion of both 32-33 split proinsulin (SP) and intact proinsulin (IP) has been shown to be an independent

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risk marker of T2DM [24,25] and is associated with coronary atherosclerosis and myocardial infarction [26,27].

The present study investigated several aspects of proinsulin secretion before and during an oral glucose tolerance test (OGTT) in white HIV-infected men with (LIPO) and without (NONLIPO) lipodystrophy, who did not differ significantly in age, duration of HIV infection, and duration and modality of HAART. The results of the present study demonstrated that LIPO patients, who predominantly exhibited impaired glucose tolerance (IGT) compared with a predominant normal glucose tolerance (NGT) in NONLIPO patients, displayed fasting and oral glucose–induced hyperproinsulinemia, increased acute secretion of IP, and increased ratio of proinsulin to insulin and C-peptide, factors that may contribute to an increased risk of CVD.

2. Materials and methods

2.1. Study subjects

The patients were recruited from the outpatient clinic at the Department of Infectious Diseases, Hvidovre University Hospital, Copenhagen, Denmark. Recruitment criteria have been described previously [5]. In brief, white men who were older than 18 years, were HIV-1–positive, and had been receiving more than 12 months of HAART were included. The patients filled in a questionnaire, which included 7 criteria of lipodystrophy (ie, loss of fat in the face, arms, legs, and buttocks, more exposed veins [lipoatrophy] and gain of fat in the abdomen/trunk, fat pads in the neck region [lipoaccumulation]), all of which should have appeared after starting HAART. Patients were physically examined for lipoatrophy in the face, extremities, and buttocks and for lipoaccumulation in the abdomen/trunk and neck. To be categorized as LIPO, the patient had to report at least one criterion of lipoatrophy and at least one criterion of lipoaccumulation. In addition, the patient had to present at least one sign of lipoatrophy and one sign of lipoaccumulation. For NONLIPO, the responses to the questionnaire about lipodystrophy as well as results of the physical examination for signs of lipodystrophy had to be negative. Criteria for exclusion for all HIV-infected patients were former diagnosis of diabetes mellitus (DM) or IGT, chronic disease other than HIV, an AIDS-related episode or an acute infection within the last 3 months, weight loss or gain greater than 4 kg within 4 months, treatment with antilipid or antidiabetic drugs, former intravenous drug abuse, current drug abuse, and participation in competitive sports. Sixteen LIPO and 15 NONLIPO patients were recruited and underwent the metabolic and body-composition measurements described below. Subjects gave their written informed consent and the protocol was approved by the ethics committee in Copenhagen, Denmark, and performed in accordance with the Helsinki Declaration II.

2.2. Oral glucose tolerance test

Patients were advised to adhere to their normal diet and to refrain from strenuous physical exercise 3 days before performing the OGTT. The patients were admitted to the clinical research center after abstinence from HIV medication for 18 hours and an overnight 12-hour fast. The standard OGTT (75 g glucose) was performed. A catheter was inserted into an antecubital vein and blood samples were drawn at −10, 0, 10, 20, 30, 45, 60, 75, 90, 105, and 120 minutes for the measurement of plasma concentrations of glucose, C-peptide, and insulin. Plasma concentration of proinsulin was measured at 0, 10, 20, 45, 75, and 105 minutes. Blood samples were centrifuged immediately at 4°C and stored at −80°C for later analysis, except for plasma glucose concentrations, which were determined immediately. Patients with a 2-hour plasma glucose level of 7.8 mmol/L or greater and less than 11.1 mmol/L were categorized as having IGT, with a 2-hour plasma glucose less than 7.8 mmol/L as having NGT, and with a 2-hour plasma glucose of 11.1 mmol/L or greater as having DM.

2.3. Body composition and anthropometry

Body composition was estimated by dual energy x-ray absorptiometry scanning (XR-36, Norland Medical Systems, 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. Peripheral fat mass was defined as the sum of arm and leg fat masses. Body weight and height were 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.

2.4. Assays

Plasma glucose concentrations were analyzed with a Beckman Analyzer (Beckman Instruments, Fullerton, CA).

Plasma insulin, C-peptide, IP, and 32-33 SP concentrations were determined by 1235 AutoDELFIA automatic fluoroimmunoassay system (Wallac, Turku, Finland). Assays for the measurement of insulin and C-peptide were supplied by Wallac; those for IP and SP were developed at the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK. The insulin assay had a detection limit of approximately 3 pmol/L. Cross-reactivity was 0.1%, 0.4%, and 66% with IP, 32-33 SP, and 64-65 SP, respectively. There was no detectable cross-reactivity with C-peptide. Typical intra- and interassay coefficients of variation (CVs) of 4.5% and 7%, respectively, were achieved. Detection limit of the C-peptide assay was approximately 5 pmol/L. Cross-reactivity was 51%, 35%, and 92% with IP, 32-33 SP, and 64-65 SP, respectively; there was no detectable cross-reactivity with insulin. The concentration of 64-65 SP is less than 7% of that of 32-33 SP in patients with a broad range of insulin sensitivity [28]

Table 1
Characteristics of study groups

	LIPO	NONLIPO	*P*
No. of patients (male)	16	15	
Age (y)	51 (2)	45 (3)	NS
Body mass index (kg/m^2)	25.0 (0.7)	22.9 (0.8)	NS
Total fat mass (% of BW)	20.3 (1.3)	18.0 (1.7)	NS
PF/TF (%)	55 (3)	94 (7)	<.001
Waist-hip ratio	1.00 (0.02)	0.91 (0.01)	.001
Fp total cholesterol (mmol/L)	6.5 (0.3)	5.5 (0.3)	<.05
Fp HDL cholesterol (mmol/L)	0.95 (0.12)	0.97 (0.11)	NS
Fp triglyceride (mmol/L)	3.5 (2.8-5.5)	2.8 (1.2-4.0)	NS
Fp creatinine (μmol/L)	84 (4)	88 (3)	NS
CD4 ($cells/mm^3$)	545 (52)	371 (53)	<.05
HIV RNA ($copies/mm^3$)	<20 (<20-472)	<20 (< 20-1590)	NS
Duration of HIV infection (mo)	111 (15)	83 (13)	NS
Duration of NRTI therapy (mo)	52 (8)	48 (7)	NS
Duration of PI therapy (mo)	36 (4)	26 (4)	NS

Values are expressed as mean (SEM) or median (interquartile range). NS indicates not significant; BW, bodyweight; Fp, fasting plasma.

and, therefore, the significance of the cross-reactivity between 64-65 SP to insulin and C-peptide is considered to be limited. Typically, intra-assay and interassay CVs of 5% and 8%, respectively, were achieved. IP and 32-33 SP were assayed in duplicate by using two-step, time-resolved fluorometric assays, adaptations of assays described previously [28]. The IP assay shows less than 0.05% cross-reaction with insulin and less than 1% cross-reaction with 32-33 SP at concentrations of 6000 and 400 pmol/L, respectively. Total imprecision (expressed as CV) was 8.2%, 6.6%, and 4.9% at 6.2, 13.7, and 81.1 pmol/L, respectively. The 32-33 SP assay shows 100% cross-reaction with IP. To obtain a specific measure of 32-33 SP it is necessary to take account of the IP concentration of the specimen. Cross-reaction with insulin is less than 0.05% at 6000 pmol/L. Total imprecision (CV) was 7.6%, 6.5%, and 3.9% at 6.3, 14.0, and 79.9 pmol/L, respectively. The limit of detection of the IP and the 32-33 SP assay was less than 1.25 pmol/L. In total, 184 (>98%) of a potential 186 samples were analyzed for IP and 181 (>97%) of 186 samples for 32-33 SP. Missed samples were due to shortage of aliquots.

Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and serum triglyceride levels were determined by reflection photometry (Ortho-Clinical Diagnostics kit, Raritan, NJ) with typical interassay CVs, across the measured ranges, of 2%, 8%, and 2.5%, respectively. Plasma lactate was quantified on an automatic lactate analyzer (ABL-735 analyzer system, Radiometer, Copenhagen, Denmark). CD4 count determination (flow cytometry, Becton-Dickinson FACscan, BD, Franklin Lakes, NJ; interassay CV, 7%) and viral load determination (Roche Amplicor and Roche amplicer ultrasensitive assay with a detection limit of 20 copies per milliliter plasma; Roche, Basel, Switzerland) met the requirements of interlaboratory quality control.

2.5. Calculations

The areas under the curve (AUCs) were calculated by the trapezoidal rule. Short-term response of proinsulin to oral glucose was defined arbitrarily as incremental secretion from 0 to 10 minutes during the OGTT. Proinsulin secretion during the early phase of the OGTT was defined arbitrarily as AUC 0 to 20 minutes ($AUC_{0\text{-}20min}$) and during the late phase as AUC 45 to 105 minutes ($AUC_{45\text{-}105min}$). Prehepatic insulin secretion rates (ISRs) were calculated from plasma C-peptide concentrations by using the ISEC (insulin secretion) computer program [29]. To derive insulin sensitivity from OGTT data we calculated the insulin sensitivity index ($ISI_{composite}$) suggested by Matsuda and DeFronzo [30] for the OGTT. $ISI_{composite}$ has been shown to correlate closely with the *M* value of the glucose clamp in individuals who display a range of glucose tolerance from normal to frank DM [30].

2.6. Statistical methods

Data are presented as mean ± SEM or median (interquartile range), and group differences were tested by unpaired *t* tests or Mann-Whitney test when appropriate. Pearson or Spearman correlation coefficients were calculated when appropriate. Calculations were performed by SPSS version 12.0 (SPSS, Chicago, IL). Two-sided *P* values less than .05 were defined as statistically significant.

3. Results

3.1. Characteristics of the study groups

A detailed description of the study population including data on glucose metabolism and prehepatic insulin secretion has been presented previously [5]. LIPO patients displayed a 36% increase in trunk fat mass (TF) and a 15% decrease in peripheral fat mass (PF; after correction for body weight), which resulted in a highly significant lower PF/TF ratio (%) in LIPO compared with NONLIPO (Table 1). Age and body mass index were not significantly different between groups. LIPO patients displayed higher total cholesterol and CD4 count than NONLIPO patients. Duration of HIV infection and duration of antiretroviral therapy did not differ significantly between study groups. In addition, components of the current antiretroviral treatment appeared balanced between LIPO and NONLIPO, that is, nucleoside reverse transcriptase inhibitors (NRTIs; any 100% vs 100%): lamivudine, 81% vs 80%; stavudine, 56% vs 33%;

Table 2
Glucose metabolic data including proinsulin release

	LIPO	NONLIPO	*P*
No. of subjects	16	15	
Fp glucose (mmol/L)	5.9 (0.2)	5.5 (0.2)	NS
2-h plasma glucose (mmol/L)	9.1 (7.1-10.2)	6.1 (5.8-7.6)	<.001
No. of NGT/IGT/DM	6/8/2	12/2/1	.05[a]
% IGT and DM	63	20	.02[a]
Fp insulin (pmol/L)	71 (57-113)	37 (21-63)	<.001
Fp C-peptide (pmol/L)	1110 (882-1463)	561 (423-873)	<.001
Fasting ISR (pmol kg^{-1} min^{-1})	3.5 (0.3)	2.2 (0.2)	<.01
$ISI_{composite}$ ($\times 10^{-4}$ $L^2 \cdot mg^{-1} \cdot \mu U^{-1}$)	3.0 (1.9-4.0)	6.8 (4.5-10.1)	<.001
Fp lactate (mmol/L)	1.8 (1.2-2.0)	1.1 (1.0-1.5)	<.01
Fp IP (pmol/L)	5.3 (3.2-11.2)	1.8 (1.3-4.2)	<.005
Fp SP (pmol/L)	11.3 (3.5-20.6)	3.2 (2.0-5.0)	<.001
Fp IP + SP (pmol/L)	17.7 (7.5-35.4)	4.5 (3.5-9.2)	<.005
$AUC_{0\text{-}20min}$ IP (pmol L^{-1} min^{-1})	11.2 (1.7)	5.1 (1.2)	<.01
$AUC_{0\text{-}20min}$ SP (pmol L^{-1} min^{-1})	18.1 (2.5)	7.7 (1.3)	<.001
$AUC_{45\text{-}105min}$ IP (pmol L^{-1} min^{-1})	41 (7)	17 (3)	<.005
$AUC_{45\text{-}105min}$ SP (pmol/L min^{-1})	61 (9)	27 (4)	<.001
Fp IP/insulin ratio (%)	7.0 (5.1-11.2)	6.1 (4.9-8.0)	NS
Fp SP/insulin ratio (%)	15.5 (8.7-22.7)	8.6 (7.0-11.4)	<.05
Fp (IP + SP)/insulin ratio (%)	24.1 (14.0-32.8)	14.6 (11.9-17.8)	<.05
Fp IP/C-peptide ratio (%)	0.50 (0.41-0.75)	0.36 (0.30-0.47)	NS
Fp SP/C-peptide ratio (%)	1.00 (0.75-1.46)	0.55 (0.42-0.73)	<.01
Fp (IP + SP)/C-peptide ratio (%)	1.57 (1.16-2.15)	1.00 (0.74-1.17)	<.05
Fp IP/ISR ratio	1.76 (1.15-2.64)	1.18 (0.94-1.38)	<.05
Fp SP/ISR ratio	3.46 (2.11-5.05)	1.49 (1.09-2.27)	<.01
Fp (IP + SP)/ISR ratio	5.49 (3.24-7.65)	2.81 (2.03-3.47)	<.01
Fp SP/IP ratio (%)	195 (16)	146 (12)	<.05
% of increased IP[b]	75[†]	33	<.02[a]
% of increased SP[b]	69[†]	13	<.01[a]
Acute incremental IP (pmol/L)[c]	3.6 ± 0.6	1.4 ± 0.2	<.005
Acute incremental SP (pmol/L)[c]	3.2 ± 1.1	2.3 ± 0.8	NS
Acute incremental IP + SP (pmol/L)[c]	6.8 ± 1.4	3.7 ± 1.0	NS
Acute incremental ISR (pmol min^{-1} kg^{-1})[c]	4.5 ± 1.2	3.8 ± 0.9	NS

Values are expressed as mean (SEM) or median (interquartile range). Fp indicates fasting plasma.
[a] χ^2 test.
[b] Upper normal tertiles defined as IP >3.0 pmol/L and SP >7.1 pmol/L [25].
[c] Increments were calculated from fasting and 10-minute values during an OGTT.
[†] $P < .01$ compared with the distribution of a normal reference group [25].

zidovudine, 38% vs 53%; didanosine, 6% vs 13%; abacavir, 6% vs 0%; protease inhibitors (PIs; any 88% vs 93%): indinavir, 44% vs 20%; ritonavir, 25% vs 40%; nelfinavir, 25% vs 27%; saquinavir, 19% vs 7%; and non-NRTIs (any 0% vs 33%): nevirapine, 0% vs 20%; efavirenz, 0% vs 13%.

3.2. Glucose metabolic data including proinsulin

Fasting glucose was similar between LIPO and NON-LIPO patients, whereas 2-hour plasma glucose during the OGTT was found increased in LIPO; accordingly, more

Table 3
Correlations between proinsulin vs glucose tolerance and insulin sensitivity

	2-h PG	$ISI_{composite}$	Fp insulin	$AUC_{0\text{-}20min}$[a]	$AUC_{45\text{-}105min}$[a]
Fp IP	0.62**	−0.86**	0.91**	0.93**	0.73**
Fp SP	0.63**	−0.85**	0.92**	0.92**	0.81**
Fp IP + SP	0.66**	−0.86**	0.92**	0.91**	0.80**
$AUC_{0\text{-}20min}$ IP	0.57**	−0.83**			
$AUC_{0\text{-}20min}$ SP	0.50*	−0.81**			
$AUC_{45\text{-}105min}$ IP	0.56*	−0.79**			
$AUC_{45\text{-}105min}$ SP	0.49*	−0.82**			

Spearman correlation coefficients are indicated. N = 31. PG indicates plasma glucose; Fp, fasting plasma.
[a] Correlation between AUC of the proinsulin (given in the left column) vs the fasting value of that specific proinsulin.
* $P < .01$.
** $P < .001$.

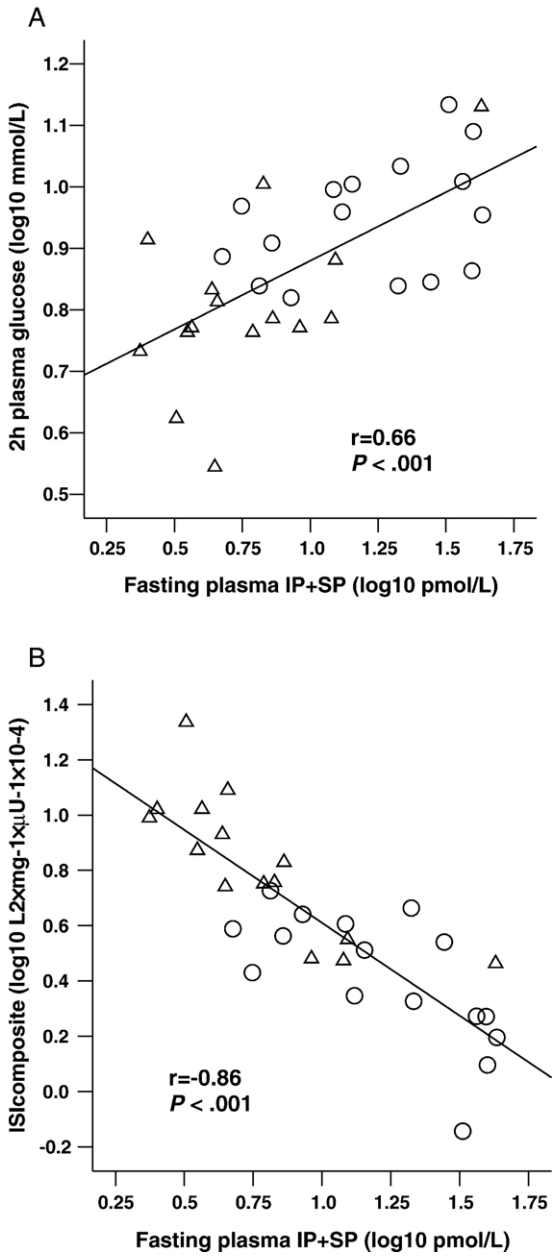

Fig. 1. Correlation (Pearson) (A) between total proinsulin (IP + SP) vs 2-hour plasma glucose during an OGTT (75 g) and (B) between total proinsulin (IP + SP) vs $ISI_{composite}$ derived from insulin and glucose values during the OGTT (reference). (○) LIPO patients (n = 16); (△) NONLIPO patients (n = 15).

patients had IGT and DM in the LIPO group compared with the NONLIPO group (Table 2). Fasting insulin, C-peptide, prehepatic insulin secretion, and plasma lactate levels were highly significantly increased and insulin sensitivity was decreased in LIPO. IP and SP were increased during fasting and early ($AUC_{0\text{-}20min}$) and late phases of the OGTT ($AUC_{45\text{-}105min}$) in the LIPO group. Ratios of fasting plasma IP, 32-33 SP, and total proinsulin (IP + SP) to insulin, C-peptide, and ISR were all increased in LIPO compared with NONLIPO patients and, with the exception of IP/insulin and IP/C-peptide, the differences were all significant.

It appeared that approximately 3 times as many LIPO patients as NONLIPO patients showed increased fasting IP and SP levels by use of a reference cohort [25] consisting of white HIV-negative, apparently healthy men with a mean age of 50 years, in whom fasting plasma proinsulin concentrations were measured using identical assays and methodology as in the present study. In addition, LIPO patients were hyperproinsulinemic compared with this reference cohort. The acute secretion of IP was more than 2.5-fold greater in LIPO compared with NONLIPO, whereas prehepatic insulin secretion did not differ between groups.

Correlations between fasting IP, SP, total proinsulin (IP + SP), and $AUC_{0\text{-}20min}$ and $AUC_{45\text{-}105min}$ of both IP and SP correlated strongly with glucose tolerance and insulin sensitivity (Table 3). In addition, fasting IP and SP appeared to be strong predictors of both $AUC_{0\text{-}20min}$ and $AUC_{45\text{-}105min}$ of IP and SP. The highly significant linear correlations between total fasting proinsulin (IP + SP) vs 2-hour plasma glucose (positive) and total fasting proinsulin vs $ISI_{composite}$ (inverse) are shown in Fig. 1. LIPO patients showed an approximately 1.5-fold greater ratio of total proinsulin to C-peptide compared with NONLIPO patients at all measurement points during the OGTT (Fig. 2). Notably, NGT LIPO (n = 6) demonstrated increased IP and SP compared with NGT NONLIPO (n = 12) during the OGTT (Fig. 3).

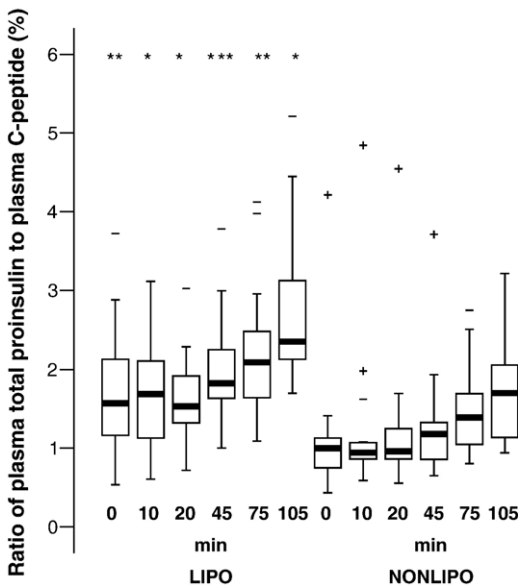

Fig. 2. Box plot of the ratios of plasma total proinsulin (IP + SP) to plasma C-peptide indicated for each time point during the OGTT at which these values were measured. Left, LIPO; right, NONLIPO. ⁻Cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box; ⁺cases with values exceeding 3 box lengths. The box length is the interquartile range. *$P < .05$, **$P < .01$, for comparison between LIPO and NONLIPO.

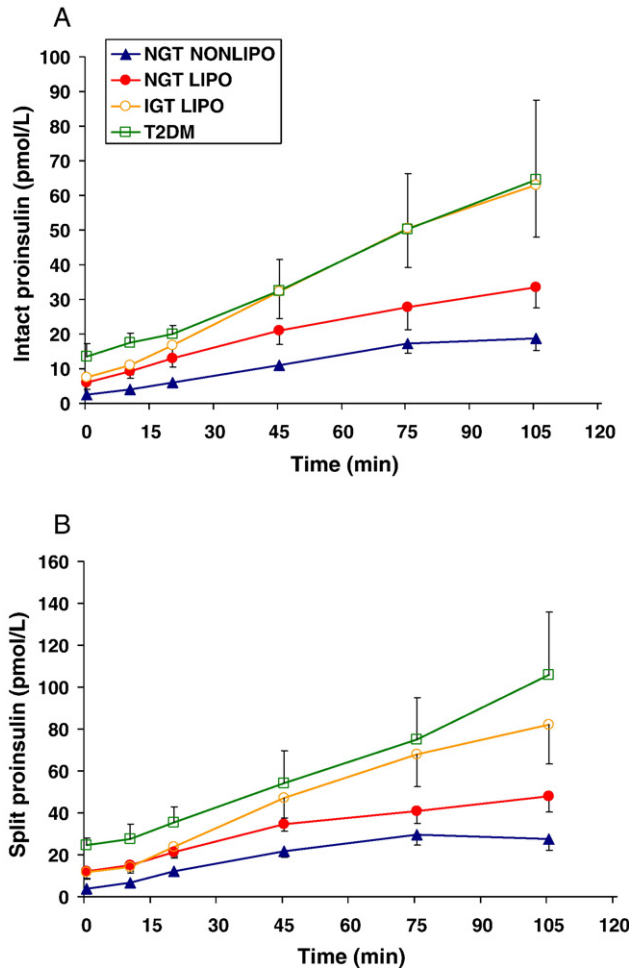

Fig. 3. IP (A) and SP (B) measured at 0, 10, 20, 45, 75, and 105 minutes during an OGTT in HIV-infected patients on HAART. (▲) NGT NONLIPO patients (n = 12); (●) NGT LIPO patients (n = 6); (○) LIPO patients who displayed IGT (n = 8); (□) patients with T2DM (LIPO n = 2; NONLIPO, n = 1). For both IP and SP, comparison between NGT LIPO and NGT NONLIPO at all time points showed $P < .05$ except at 75 minutes at which a trend was shown ($P < .2$). All comparisons between IGT LIPO and NGT NONLIPO were significantly different for both IP and SP ($P < .05$). Mean (SEM) values are given.

4. Discussion

The present study provides new insight into the secretion pattern of SP and IP in LIPO patients: normal glucose tolerant LIPO patients displayed increased fasting and oral glucose–stimulated proinsulinemia compared with their normal glucose tolerant NONLIPO counterparts. Hyperproinsulinemia was far more prevalent in LIPO compared with that of a normal reference distribution and with NONLIPO. Acute secretion of IP and fasting SP/IP and SP/insulin ratios were increased in LIPO. Fasting hyperproinsulinemia and hyperproinsulinemia during the early and late phase of an OGTT were strong predictors of both IGT and insulin resistance in HIV-infected patients on HAART.

Fasting and oral glucose–stimulated hyperproinsulinemia has been shown in HIV-infected patients under PI therapy compared with patients not taking PIs [31]. This was taken as proof that PI therapy may induce aberration in insulin processing and secretion. We studied HIV-infected patients discordant for lipodystrophy who were treated with a similar combination of antiretroviral agents. These patients were studied after more than 18 hours' discontinuation of antiretroviral therapy, which likely may have significantly attenuated the short-term effect of this therapy on insulin secretion and glucose metabolism [32,33]. Therefore, the present study may have addressed primarily the effect of the lipodystrophic phenotype on proinsulin secretion before and after oral glucose stimulation, rather than an effect of HAART per se. It may be debated whether PIs as such influence proinsulin metabolism; for example, a prospective study did not reveal any PI effect on proinsulin secretion after 12 weeks on PI [17]. In addition, in vitro evidence supports the view that PIs do not affect proinsulin processing in beta-cell cultures [34]. The present data support the interpretation that the dysmetabolism including fat redistribution of LIPO patients and not the PIs themselves is a major cause of the increased proinsulin concentration in these patients.

Plasma proinsulin clearance does not differ between individuals with or without T2DM [35], whereas plasma insulin clearance is very much dependent on insulin sensitivity in both HIV-negative [36,37] and HIV-positive individuals [38], such that systemic clearance rate and level of liver extraction of insulin are positively correlated with insulin sensitivity. The present study evaluated ratios of total proinsulin to C-peptide during the OGTT because clearance of plasma C-peptide may be only weakly associated with insulin sensitivity of the individual [39], its kinetics is linear over a wide range of plasma concentrations almost similar to that of proinsulin, and, finally, C-peptide does not show first-pass clearance in the liver, which in practice also accounts for proinsulin [40]. Notably, LIPO patients showed increased ratios of proinsulin to C-peptide at all time points during the OGTT compared with NONLIPO patients, thus inappropriately demasking fasting- and oral glucose–induced proinsulin secretion rates in LIPO patients.

Kinetic studies of proinsulin secretion have shown that SP clearance differs somewhat from that of IP, both of which, however, differ considerably from that of insulin [40]. Therefore, the proinsulin-insulin ratio in plasma provides an accurate estimate of the proinsulin-insulin ratio in the secretory granule only after short-term stimulation of insulin secretion, when differences in elimination kinetics have a negligible influence on concentrations [41-43]. Accordingly, in the present study a short-term response was defined as incremental proinsulin secretion during the initial 10 minutes after glucose ingestion. Moreover, to account more comprehensively for these differences in elimination kinetics, acute incremental proinsulin secretion was compared with acute incremental prehepatic insulin secretion derived from C-peptide concentrations. It was found that LIPO patients displayed increased acute IP secretion compared with NONLIPO patients despite a similar acute prehepatic insulin

secretion between study groups. These observations, in combination with the fact that LIPO patients displayed increased fasting SP/IP ratio, as previously demonstrated in T2DM [23], support evidence that LIPO patients exhibit incomplete processing of proinsulin to insulin in beta cells compared with NONLIPO patients.

In HIV-negative patients with IGT or manifest T2DM, the insulin response during an OGTT is delayed and always reduced when related to the ambient insulin resistance [44]. An increased proinsulin-insulin ratio is also a well-established abnormality in subjects with IGT and T2DM and is related to the degree of impairment in beta-cell function [44,45]. In healthy subjects, insulin and proinsulin release increases with insulin resistance, but this alteration occurs in parallel, so that the proinsulin-insulin ratio does not change [46]. Thus, fasting proinsulin level reflects the degree of insulin resistance. In contrast, in subjects with normal plasma glucose levels who subsequently develop T2DM the proinsulin to insulin ratio was elevated [47]. Therefore, an elevated proinsulin-insulin ratio predicts the subsequent development of T2DM and is an early indicator of beta-cell secretory insufficiency.

Our previous results of hyperproinsulinemia in normoglycemic LIPO compared with normoglycemic NONLIPO [48] comply with the present findings that normal glucose tolerant LIPO patients displayed hyperproinsulinemia before and after oral glucose stimulation compared with their NONLIPO counterparts. Thus, despite normal fasting plasma glucose and normal 2-hour plasma glucose during an OGTT, LIPO patients exhibit aberration in proinsulin secretion. This observation adds to the argument that LIPO patients should undergo rigorous surveillance of glucose homeostasis for facilitating early intervention to hinder further deterioration in beta-cell function and to prevent DM [12]. Screening should be performed by fasting plasma glucose determination followed by an OGTT in people with a fasting plasma glucose concentration greater than 5.6 and less than 7.0 mmol/L. World Health Organization 1999 criteria should be used to diagnose diabetes [49].

In the present study both glucose tolerance (2-hour plasma glucose after oral glucose) and insulin sensitivity correlated strongly with postabsorptive and glucose-stimulated proinsulin secretion. Impaired glucose tolerance and insulin resistance are strong predictors of the development of DM in the general population [50]. Diabetes is strongly associated with ischemic heart disease [51], apparently also in HIV-infected patients on HAART [14]. Therefore, a future perspective might be to investigate whether the level of proinsulinemia in HIV-infected patients is associated with coronary atherosclerosis and myocardial infarction as has been demonstrated in the general population [26,27].

A limitation of the present study, besides being cross-sectional and therefore cannot determine causal relationships, is that a normal reference group was not included. However, comparison with historical data generated using identical assays of proinsulin demonstrated elevated fasting plasma proinsulin levels in LIPO, whereas this was normal in NONLIPO. It should be emphasized that we aimed to study primarily the effect of HIV lipodystrophy on proinsulin release; therefore, a group of NONLIPO patients may be the most relevant control group.

In conclusion, the present study demonstrated a dysregulation of proinsulin secretion during fasting and after oral glucose stimulation in HIV lipodystrophy. Given the strong predictive value of hyperproinsulinemia for T2DM and CVD in the general population, initiatives should be taken to identify LIPO patients to improve glucose metabolism and to address other CVD risk factors inherent in this clinical state. Large prospective studies are warranted to investigate the value of hyperproinsulinemia to predict metabolic and cardiovascular events in HIV-infected patients receiving HAART.

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