Insulin-Like Growth Factors, Insulin-Like Growth Factor-Binding Proteins, Insulin-Like Growth Factor-Binding Protein-3 Protease, and Growth Hormone-Binding Protein in Lipodystrophic Human Immunodeficiency Virus-Infected Patients

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Human immunodeficiency virus (HIV)-lipodystrophy is associated with impaired growth hormone (GH) secretion. It remains to be elucidated whether insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), IGFBP-3 protease, and GH-binding protein (GHBP) are abnormal in HIV-lipodystrophy. These parameters were measured in overnight fasting serum samples from 16 Caucasian males with HIV-lipodystrophy (LIPO) and 15 Caucasian HIV-infected males without lipodystrophy (NONLIPO) matched for age, weight, duration of HIV infection, and antiretroviral therapy. In LIPO, abdominal fat mass and insulin concentration were increased (>90%, $P < .01$) and insulin sensitivity ($Log10ISI_{composite}$) was decreased (−50%, $P < .001$). Total and free IGF-I, IGF-II, IGFBP-3, and IGFBP-3 protease were similar between groups (all $P > .5$), whereas, in LIPO, IGFBP-1 and IGFBP-2 were reduced (−36%, $P < .05$ and −50%, $P < .01$). In pooled groups, total IGF-I, free IGF-I, total IGF-II, and IGFBP-3, respectively, correlated inversely with age (all $P < .01$). In pooled groups, IGFBP-1 and IGFBP-2 correlated positively with insulin sensitivity (age-adjusted all $P < .05$). IGFBP-3 protease correlated with free IGF-I in pooled groups ($r_p = 0.47$, $P < .02$), and in LIPO ($r_p = 0.71$, $P < .007$) controlling for age, total IGF-I, and IGFBP-3. GHBP was increased, whereas GH was decreased in LIPO (all $P < .05$). GH correlated inversely with GHBP in pooled groups ($P < .05$). Taken together the similar IGFs and IGFBP-3 concentrations between study groups, including suppressed GH, and increased GHBP in LIPO, argue against GH resistance of GH-sensitive tissues in LIPO compared with NONLIPO; however, this notion awaits examination in dose-response studies. Furthermore, our data suggest that IGFBP-3 protease is a significant regulator of bioactive IGF-I in HIV-lipodystrophy.

HUMAN IMMUNODEFICIENCY virus (HIV)-infected patients with fat redistribution of the mixed type (LIPO, ie, peripheral fat loss and central fat gain)[1] display impaired growth hormone (GH) secretion compared with HIV-infected patients without fat redistribution (NONLIPO) and healthy individuals.[2,3] The aberrations in GH secretion include decreased GH mean peak amplitude, mean secretion mass, and baseline concentration.[3] GH induces and increases production of insulin-like growth factor I (IGF-I) and IGF binding protein-3 (IGFBP-3) in man.[4] It may be hypothesized, therefore, that LIPO display reduced circulating levels of IGF-I and IGFBP-3, as has been described in states of GH deficiency.[5,6] Alternatively, if serum concentrations of GH-sensitive IGFs are similar or increased in LIPO compared with NONLIPO, LIPO would likely exhibit enhanced GH sensitivity of tissues producing and secreting GH-sensitive IGF, as has been demonstrated in obesity.[7,8] Levels of GH-binding protein (GHBP), which is accepted by some as a marker of GH receptor sensitivity,[9] might elucidate this issue.

In the circulation, the majority of IGF-I is bound to IGFBP-3[10] with less than 1% of IGF-I found in a free fraction.[11] Increased IGFBP-3 proteolytic activity may be an important regulator of IGF-I bioactivity.[12] Increased IGFBP-3 proteolysis has been reported in insulin-resistant states[12,13] and in states of impaired GH secretion.[14] Accordingly, lipodystrophic HIV-infected patients, who are characterized by insulin resistance and impaired GH secretion, may display an increased level of IGFBP-3 proteolysis.

We aimed to investigate whether the total and free IGF-I, -II, IGFBP-1, -2, -3, and IGFBP-3 protease activity, including GHBP and fasting GH concentrations, were abnormal in a group of lipodystrophic HIV-infected patients who display insulin resistance and increased abdominal fat mass compared with an age- and weight-matched group of HIV-infected patients without lipodystrophy.

MATERIALS AND METHODS

Study Subjects

The patients were recruited from the outpatient clinic at the Department of Infectious Diseases, Hvidovre Hospital, University of Copenhagen, Denmark. We included Caucasian males, who were older than 18 years, had a positive HIV-1 antibody test, had been receiving highly active antiretroviral therapy (HAART) for more than 12 months, and who either complained (LIPO) or did not complain (NONLIPO) of changes in fat distribution. The patients were asked to fill out a questionnaire, which included 7 criteria of lipodystrophy (ie, for lipoatrophy: loss of fat in face, arms, legs, buttocks, more exposed veins, and for lipoaccumulation: gain of fat at abdomen/trunk, fat pads in the neck region). A trained physician performed all the physical examinations (examination for lipoatrophy in face, extremities, buttocks, and for

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lipoaccumulation in abdomen/trunk and buffalo hump). To be categorized as LIPO, the patient had to report at least 1 criterion of lipoatrophy and at least 1 criterion of lipoaccumulation. In addition, the patient had to present with at least 1 sign of lipoatrophy and 1 sign of lipoaccumulation. For NONLIPO, the questionnaire about lipodystrophy, as well as the physical examination for signs of lipodystrophy, had to be negative. Criteria for exclusion for all HIV-infected patients were previously diagnosed diabetes mellitus or impaired glucose tolerance,[15] chronic disease other than HIV, an acquired immune deficiency syndrome (AIDS)-related episode or an acute infection within the latest 3 months, weight loss or gain above 4 kg within 4 months, treatment with antilipid or antidiabetic drugs, and participation in competitive sports. Sixteen lipodystrophic HIV-infected patients and 15 HIV-infected patients without lipodystrophy 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 Ethical Committee in Copenhagen, Denmark and performed in accordance with the Helsinki Declaration II.

Study Protocol, Blood Sampling, and Body Composition

Three days before performing the oral glucose tolerance test (OGTT), patients were instructed to not alter their normal diet and to refrain from strenuous physical exercise. The patients were admitted to the clinical research center at 8 AM following abstinence from HIV medication for 18 hours and an overnight 12-hour fast. A catheter was inserted in an antecubital vein. The standard OGTT (75 g) was performed as described elsewhere.[15] Blood samples were drawn at −10, 0, 30, 60, 90, and 120 minutes (relative to ingestion of glucose at 8:30 AM) for the measurement of plasma concentrations of glucose, insulin, and free fatty acids (FFA). 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 ≥ 7.8 mmol/L and <11.1 mmol/L were categorized as having impaired glucose tolerance (IGT). Patients with a 2-hour plasma glucose < 7.8 mmol/L had normal glucose tolerance (NGT), and patients with a 2-hour plasmas glucose > 11.1 mmol/L had diabetes.[16]

Body composition was estimated by dual energy x-ray absorptiometry (DEXA) scanning (XR-36; Norland Medical System, Fort Atkinson, WI), using software version 2.1.0. A whole-body scan was performed to estimate the amount of fat in the trunk, abdomen, and extremities. The trunk was defined as the region including the chest, abdomen, and pelvis. The proximal limitations of the leg regions, defined by a line through the hip joints at an angle of 45°, and for the arm regions by a line vertically through the shoulder joints. The area between horizontal lines at the levels of the xiphoid process and the iliac crest was defined as the abdominal region. Limb fat mass was defined as the sum of arm and leg fat masses. 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. To assess the distribution of visceral abdominal adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAT) a single-slice computed tomography (CT) scan at the level of L4 was performed in 14 LIPO and in 12 NONLIPO subjects. The area of adipose tissue was measured in square centimeters.

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.

Assays

Serum total IGF-I and total IGF-II were measured by an in-house noncompetitive, time-resolved immunofluorometric assay after acid ethanol extraction of serum, as previously described.[17] The within assay coefficient of variation (CV) of total IGF-I and total IGF-II averages less than 5%. Serum free IGF-I and free IGF-II were measured by ultrafiltration, as previously described.[11] Including ultrafiltration and immunoassay, the within assays CV averaged 18% and 12% for free IGF-I and free IGF-II, respectively. Serum IGFBP-3 was measured by immunoradiometric assay (IRMA, Diagnostic Systems Laboratories, Webster, TX). Serum IGFBP-1, -2, and -3 were analyzed by Western ligand blotting (WLB). WLB, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and ligand blot analysis were performed in serum according to the method of Hossenlopp et al[18] as previously described.[19] Two microliter serum was subjected to SDS-PAGE (10% polyacrylamide) under nonreducing conditions.

The ^{125}I-IGFBP-3 degradation assay was performed as previously described.[20] ^{125}I-IGFBP-3 (30,000 cpm) (Diagnostic Systems Laboratories) was incubated for 18 hours at 37°C. Two microliter serum from the HIV-infected patients was subjected to SDS-PAGE as described above. On each gel, serum samples from healthy nonpregnant subjects and term-pregnant women were used as internal controls. Gels were fixed in a solution of 7% acetic acid, dried, and autoradiographed. The degree of proteolysis was calculated as a ratio of the absorbency of fragmented ^{125}I-IGFBP-3 over the sum of all ^{125}I-IGFBP-3–related optical densities in that lane and was expressed as a percentage. The in-between assay CV of the control samples averaged 10%.

GH levels were determined by an immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland).[21] GHBP was determined by an immunofunctional time-resolved fluoroimmunoassay as described previously.[21]

Plasma glucose concentrations were analyzed with a Beckman Analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin and C-peptide concentrations were determined by AutoDELPHIA automatic fluoroimmunoassay system (Wallac Oy). The insulin assay had a detection limit of approximately 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 approximately 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 CV of 5% and interassay CV of 8%. Plasma FFA were determined using an enzymatic colorimetric method (Wako C test kit; Wako Chemicals GmbH, 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. CD4 count determination (flow cytometry, Becton-Dickinson FACscan, BD, Frankling 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

Ratio of limb fat (RLF) was calculated as peripheral fat mass divided by trunk fat mass and used as an indicator of fat distribution.

We calculated the insulin sensitivity index suggested by Matsuda and DeFronzo[22] for the OGTT, denoted $ISI_{composite}$:

$$ISI_{composite} = \frac{10{,}000}{\sqrt{FPG \cdot FPI \cdot MG_{0\text{-}120} \cdot MI_{0\text{-}120}}}$$

FPG and FPI are the fasting plasma glucose and insulin concentrations, respectively. $MG_{0\text{-}120}$ and $MI_{0\text{-}120}$ are the means of the glucose,

Table 1. Parameters of Anthropometry and Glucose Metabolism

	LIPO	NONLIPO	*P*
Number-gender	16 males	15 males	
Age (yr)	51 (2)	45 (3)	NS
Body mass index (kg/m^2)	25 (1)	23 (1)	NS
Total fat mass (kg) (DEXA)	16.7 (1.5)	13.4 (1.6)	NS
Abdominal fat (kg) (DEXA)	5.8 (0.6)	3.0 (0.5)	<.01
Ratio limb fat/trunk fat (%)	55 (3)	94 (7)	<.0001
Abdominal VAT (cm^2) (CT)	214 (26)†	63 (12)‡	<.001
Abdominal SAT (cm^2) (CT)	112 (14)†	107 (24)‡	NS
Waist-to-hip ratio	1.00 (0.02)	0.91 (0.01)	<.001
2-h, plasma glucose (mmol/L)	9.1 (0.5)	6.8 (0.6)	<.01
Number NGT/IGT/DM	6/8/2	12/2/1	.05*
Percentage IGT and DM	63	20	.02*
Fasting p-glucose (mmol/L)	5.9 (0.2)	5.5 (0.2)	NS
Fasting p-insulin (pmol/L)	91 (13)	40 (5)	<.01
Fasting p-C-peptide (pmol/L)	1175 (109)	653 (76)	<.001
AUC-insulin$_{0\text{-}120min}$ (nmol/L · min)	72 (12)	30 (4)	<.003
Log10 ISI$_{composite}$	0.41 (0.06)	0.82 (0.07)	<.001

NOTE. Mean (SEM) and median (range). Abdominal fat (DEXA) is assessed from the level of processus xiphoideus to the fourth lumbal spine.

Abbreviations: LIPO (NONLIPO), HIV-infected patient with (without) lipodystrophy; VAT, visceral adipose tissue; SAT, subcutanuos adipose tissue; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus; AUC, area under the curve; IS$_{composite}$, insulin sensitivity index derived from oral glucose tolerance test, NS, not significant.

*χ^2 test.

†n = 14.

‡n = 12.

respectively, insulin concentrations measured at 0, 30, 60, 90, and 120 minutes during the OGTT. The concentration of glucose is expressed in milligram per deciliter and the insulin concentration in microunit per milliliter. Unit of ISI$_{composite}$ is $L^2 \cdot mg^{-1} \cdot \mu U^{-1}$. 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 diabetes.[22]

Statistics

Data are presented as means ± SEM if not otherwise indicated. Correlations between continuously measured or calculated variables were evaluated by Pearsons linear regression analysis. Partial linear correlations coefficients (r_p) between 2 parameters were calculated given adjustment for age and other variables when warranted. Given skewed data distribution, data were log transformed before applying a *t* test and before calculation of a regression coefficient. Calculations were performed by SPSS (SPSS ver. 12.0; SPSS, Chicago, IL). Two-sided *P* values less than .05 were defined as statistically significant.

RESULTS

Characteristics of Study Groups

Age, body mass index (BMI), and total fat mass did not differ significantly between LIPO and NONLIPO (Table 1). Total abdominal fat mass was increased 2-fold in LIPO as determined by DEXA or CT scan. CT scans suggested a 3-fold increase in visceral abdominal fat in LIPO. The fat distribution was different in LIPO and NONLIPO demonstrated by a highly reduced RLF in LIPO.

Although fasting glucose concentration did not differ between LIPO and NONLIPO, fasting insulin and C-peptide were increased approximately 2-fold in LIPO. During an OGTT, LIPO displayed higher 2-hour plasma glucose concentration than NONLIPO, accordingly more LIPO than NONLIPO displayed IGT and DM. Insulin sensitivity (ISI$_{composite}$) was reduced 50% (log transformed) in LIPO compared with NONLIPO.

Compared with NONLIPO, LIPO displayed increased serum total-cholesterol concentration (6.5 ± 0.3mmol/L *v* 5.5 ± 0.3, $P < .05$), whereas serum HDL-cholesterol (0.95 ± 0.12 mmol/L *v* 0.97 ± 0.11 mmol/L, *P* = not significant [NS]), serum triglyceride (4.7 ± 0.8 mmol/L *v* 3.3 ± 0.7, *P* = NS), and plasma FFA (0.56 ± 0.05 mmol/L *v* 0.48 ± 0.05, *P* = NS) did not differ significantly between groups.

CD4 counts were increased in LIPO compared with NONLIPO (545 ± 52 cells/mm^3 *v* 371 ± 53), however, mean CD4 count for each group suggested good immunologic control in both groups, which was underlined by full suppression of HIV-RNA in LIPO and NONLIPO (median < 20 [range, < 20 to 472] copies/mL^3 *v* <20 [<20 to 1,590], *P* = NS). No significant differences were found between LIPO and NONLIPO in duration of HIV (111 ± 15 months *v* 83 ± 13) and duration of treatment with the main antiretroviral therapy classes, ie, nucleoside reverse transcriptase inhibitors (52 ± 8 months *v* 48 ± 7, *P* =NS) and protease inhibitors (PIs) (36 ± 4 months *v* 26 ± 4). As part of HAART, all patients used nucleoside reverse transcriptase inhibitors (NRTIs). Frequently used NRTIs were lamivudine (85%), zidovudine (45%), and stavudine (45%), respectively. PIs were used by 90% of the patients. Frequently used PIs were indinavir (32%), ritonavir (32%), and nelfinavir (26%). Components of HAART were similar in the 2 study groups (data not shown).

IGFs, IGFBPs, IGFBP-3 Protease, GH, and GHBP Concentrations

The overnight fasting serum concentrations of total and free IGF-I, IGF-II, IGFBP-3, and IGFBP-3 protease were similar between groups (all $P > .5$), whereas GH was decreased and GHBP increased in LIPO (Table 2). IGFBP-1 and -2 were suppressed in LIPO (Fig 1A and B). For comparison of IGFBP-3 protease, healthy nonpregnant control subjects (see Materials and Methods section) displayed an IGFBP-3 protease of 42% ± 5%, which was similar to the 2 study groups, whereas the term-pregnant women had a significantly higher IGFBP-3 protease (68% ± 7%, $P < .03$).

Linear Correlations With IGFs, IGFBPs, IGFBP-3 Protease, GH, and GHBP

In the pooled study groups, total IGF-I, free IGF-I, total IGF-II, and IGFBP-3 correlated inversely with age (Table 3). Total IGF-I correlated positively with free IGF-I and total IGF-II correlated positively with free IGF-II (Fig 2A and B). However, merely 48% of the variation of free IGF-I concentration could be explained by total IGF-I concentration (pooled study groups $r^2 = 0.48$) and merely 22% of the variation of free IGF-II concentration could be explained by total IGF-II concentration (pooled study groups $r^2 = 0.22$).

Table 2. Fasting Plasma Concentrations of IGFs, IGFBP-3, IGFBP-3 Protease, GH, and GHBP

	LIPO	NONLIPO	*P*
No.	16	15	
Total IGF-I (μg/L)	180 (14)	170 (10)	NS
Free IGF-I (μg/L)	0.60 (0.06)	0.66 (0.07)	NS
Ratio of free IGF-I (%)	0.33 (0.03)	0.38 (0.03)	NS
Total IGF-II (μg/L)	939 (59)	913 (83)	NS
Free IGF-II (μg/L)	1.21 (0.10)	1.11 (0.14)	NS
Ratio of free IGF-II (%)	0.13 (0.01)	0.13 (0.01)	NS
IGFBP-3 IRMA (μg/L)	3447 (249)	3381 (169)	NS
IGFBP-3 WLB (AU)	612 (56)	669 (105)*	NS
IGFBP-3 protease (%)	42 (2)	40 (2)*	NS
Log10 GH (ng/L)	2.11 (0.13)	2.56 (0.17)	.04
Log 10 GHBP (pmol/L)	3.27 (0.07)	3.03 (0.08)	.03

Abbreviations: IRMA, immunoradiometric assay; WLB, Western ligand blot; AU, arbitrary units.

*n = 13.

Table 3. Linear Correlation Coefficients Between Age Versus IGFs, IGFBPs, and IGFBP-3 Protease

Age	All	LIPO	NONLIPO
Total IGF-I	−0.58*	−0.67†	−0.76†
Free IGF-I	−0.56†	−0.38	−0.80*
Total IGF-II	−0.46†	−0.46	−0.53‡
Free IGF-II	−0.33	−0.33	−0.42
IGFBP-1	−0.32	0.23	−0.37
Log10 IGFBP-2	0.01	0.39	0.33
IGFBP-3 IRMA	−0.37‡	−0.60‡	−0.29
IGFBP-3 WLB	−0.43‡	−0.54‡	−0.39
IGFBP-3 protease (%)	0.50†	0.73†	0.29

Abbreviations: WLB, Western ligand blot; IRMA, immunoradiometric assay.

*$P < .001$.

†$P < .01$.

‡$P < .05$.

As IGFBP-3 is the most abundant and main binding protein for IGF-I and IGF-II, strong correlations between IGFBP-3 and IGFs were expected and demonstrated (Table 4).

In pooled study groups, controlling for age, IGFBP-1 did not correlate with free IGF-I ($P > 0.7$), whereas IGFBP-2 correlated inversely with free IGF-II after controlling for age (log-log transformed $r_p = -0.46$, $P < .02$).

IGFBP-3 measured by WLB (determines intact IGFBP-3) and IGFBP-3 measured by IRMA (determines intact plus fragmented IGFBP-3) correlated strongly, however, linearity was not complete (LIPO, $r^2 = 0.42$; NONLIPO, $r^2 = 0.53$; Fig 3A).

IGFBP-1 and IGFBP-2 correlated inversely with fasting insulin and C-peptide concentrations, whereas insulin sensitivity index correlated positively with IGFBP-1 and -2 (Table 5).

GHBP was inversely correlated with GH after log-log transformation of serum concentrations, $r = -0.40$, $P < .05$. GHBP and GH did not correlate significantly with age (data not shown). In pooled study groups, GHBP correlated significantly and positively with measures of abdominal fat mass (DEXA abdominal fat mass $r = 0.64$, $P < .001$; CT total abdominal fat area $r = 0.71$, $P < .001$; CT visceral abdominal fat area $r = 0.66$, $P < .001$), whereas GH correlated inversely with measures of total abdominal fat (DEXA $r = -0.46$, $P < .01$; CT $r = -0.41$, $P < .05$). Correction for age did not influence the significance of these correlations.

Total and free IGF-I and IGF-II did not correlate significantly with insulin sensitivity or abdominal fat mass determined by DEXA and CT scans (data not shown). CD4 count did not correlate significantly with any of the IGFs, IGFBPs, or IGFBP protease activity (data not shown).

Partial Correlations With IGFBP-3 Protease

The associations between IGFBP-3 (WLB) and IGFBP-3 protease (Fig 3B and C) remained significant after controlling for age, ie, in pooled study groups ($r_p = -0.69$, $P < .001$), in LIPO ($r_p = -0.56$, $P < .05$), and in NONLIPO ($r_p = -0.89$, $P < .001$), respectively. Thus, low intact IGFBP-3 was shown

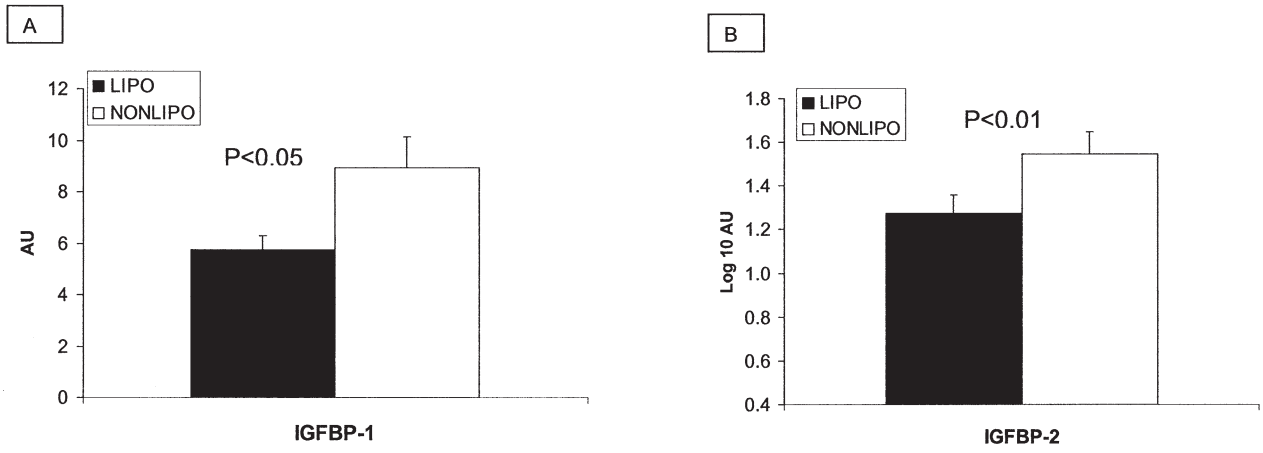

Fig 1. Fasting serum concentrations of (A) IGFBP-1 and (B) IGFBP-2 in lipodystrophic HIV-infected patients (LIPO, ■) and HIV-infected patients without lipodystrophy (NONLIPO, □). IGFBP-1 and -2 were determined in all LIPO (n = 16) and in n = 13 NONLIPO. The values are given in arbitrary units (AU) and are log transformed for IGFBP-2.

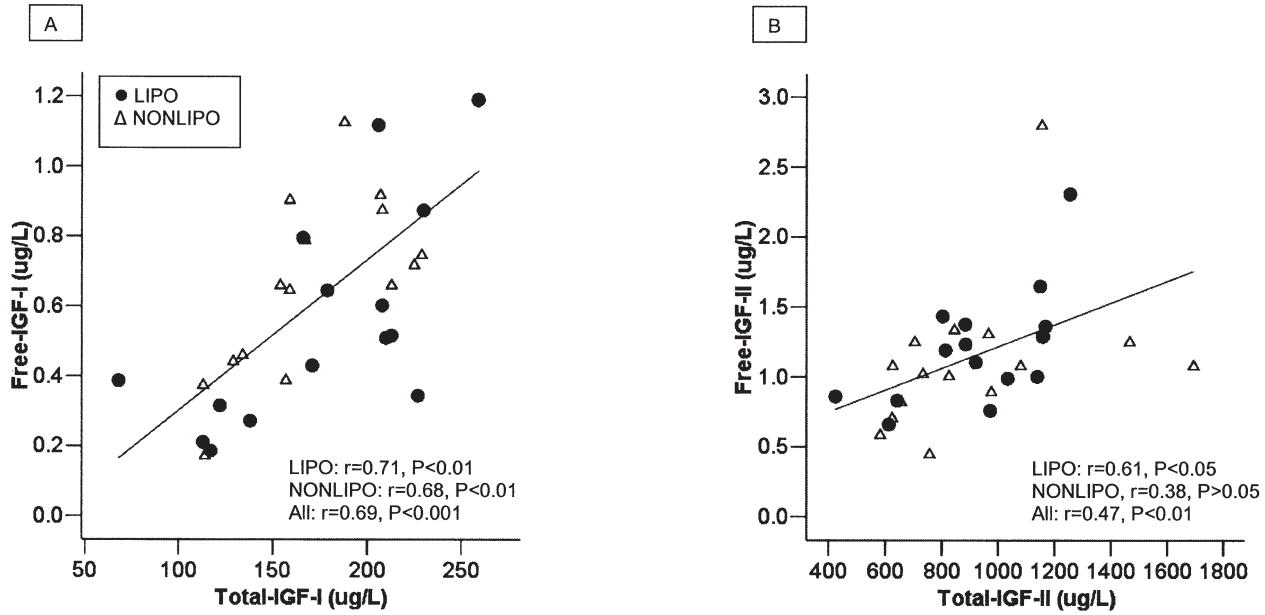

Fig 2. (A) Scatter plots of total-IGF-I *v* free IGF-I. (B) Scatter plots of total IGF-II *v* free IGF-II. ●, lipodystrophic HIV-infected patients (LIPO; △, HIV-infected patients without lipodystrophy (NONLIPO); unbroken lines, linear regression lines for pooled groups.

to be associated with high protease activity independently of age.

Accordingly, we examined whether IGFBP-3 protease plays an independent role for the bioactivity of IGF-I. In a partial linear regression analysis, controlling for the strong covariate factors of free IGF-I, ie, total IGF-I and IGFBP-3 (IRMA), IGFBP-3 protease correlated positively and significantly with free IGF-I in pooled study groups ($r_p = 0.40$, $P < .05$) and in LIPO ($r_p = 0.75$, $P < .003$), but not in NONLIPO ($P > .8$). Including age as a covariate in the analysis did not significantly change the partial linear regression coefficients between IGFBP-3 protease and free IGF-I, ie, for the pooled groups ($r_p = 0.47$, $P < .02$), for LIPO ($r_p = 0.71$, $P < .007$), and for NONLIPO ($P > .7$), respectively. Our data suggest an independent role for IGFBP-3 protease to enhance plasma concentration of bioactive IGF-I in HIV-infected patients on HAART, at least those who display lipodystrophy. Similar calculations substituting total IGF-I and free IGF-I with total IGF-II and free IGF-II showed a borderline significant positive correlation between IGFBP-3 protease and free IGF-II in pooled study groups when controlling for total IGF-II and IGFBP-3 ($r_p = 0.36$, $P = .065$), which was not influenced by adjustment for age ($r_p = 0.37$, $P = .065$).

Table 4. Correlation Coefficients Between IGFBP-3 (IRMA) Versus IGFs

	IGFBP-3 (IRMA)					
	Unadjusted			Age adjusted		
	L	NL	All	L	NL	All
Total-IGF-I	0.83*	0.43	0.71*	0.72†	0.34	0.65*
Free IGF-I	0.83*	0.11	0.60*	0.82*	−0.20	0.50†
Total-IGF-II	0.90*	0.48	0.66*	0.88*	0.40	0.60†
Free IGF-II	0.65†	0.18	0.41‡	0.59‡	0.06	0.33

Abbreviations: IRMA, immunoradiometric assay; L, LIPO; NL, NONLIPO.

*$P < .001$.

†$P < .01$.

‡$P < .05$.

DISCUSSION

The major observations in this descriptive study of the GH/IGF-axis in relative insulin-resistant and glucose intolerant lipodystrophic HIV-infected patients include similar serum concentrations of total and free IGF-I and IGF-II, IGFBP-3, and IGFBP-3 protease between such patients and age- and weight-matched relative insulin-sensitive and glucose tolerant HIV-infected patients without lipodystrophy. Furthermore, HIV-infected, lipodystrophic, patients displayed suppression of the predominantly insulin-sensitive IGFBP-1 and IGFBP-2, whereas GHBP was increased. Finally, our data suggest that IGFBP-3 protease, in particular in lipodystrophic patients, plays a role for IGF-I bioactivity. Beneath we will interpret these data.

IGF-I is the most important anabolic growth factor and plays its physiologic role in many tissues.[23] Circulating IGF-I is primarily produced in the liver and is GH-dependent.[24] Accordingly, in GH deficiency (GHD), IGF-I serum concentration is low.[4] However, in obesity, where GH secretion is blunted, the IGF-I concentration is normal or increased.[7,8] This might be explained by an increased sensitivity of the IGF-I producing tissue in the liver probably reflecting a compensatory mechanism.[7,25] Previously, in the era of HIV wasting (ie, before HAART was introduced), HIV-infected patients with a pronounced weight loss and immune failure demonstrated GH hypersecretion and low serum IGF-I concentrations, which was

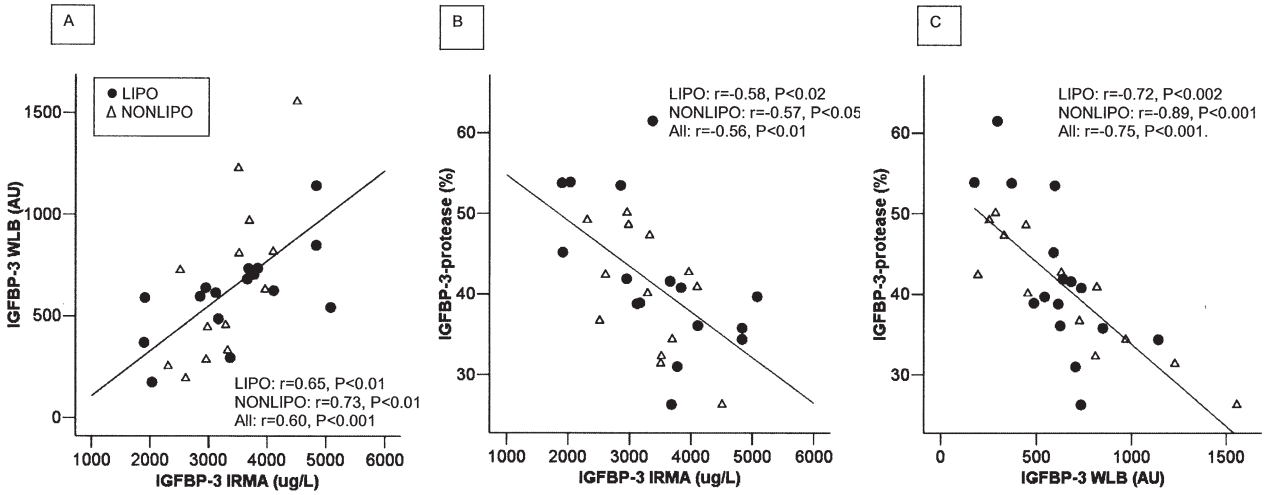

Fig 3. (A) Scatter plots of IGFBP-3 (assessed by IRMA) *v* IGFBP-3 (assessed by WLB). Values for WLB are given AU. (B) Scatter plots of IGFBP-3 (IRMA) *v* IGFBP-3 protease. (C) Scatter plots of IGFBP-3 (WLB) *v* IGFBP-3 protease. ●, lipodystrophic HIV-infected patients (LIPO); △, HIV-infected patients without lipodystrophy (NONLIPO); unbroken lines, linear regression lines for pooled groups.

interpreted as GH resistance.[26] The HIV wasting associated with GH-resistance might be explained by undernutrition,[26] although acquired resistance to GH is suggested by a decreased IGF-I response to exogenous GH[27] wherein immunologic status may play a role.[28] In the present study, a group of insulin-resistant, relatively glucose intolerant and abdominal obese normal-weight lipodystrophic HIV-infected patients had similar serum IGF-I concentrations as a group of age- and weight-matched HIV infected patients without fat redistribution. As the latter group of patients has been shown to display normal GH secretion in opposition to the impaired GH secretion in lipodystrophic HIV-infected patients,[3] lipodystrophic HIV-infected patients would likely exhibit enhanced GH sensitivity of IGF-I producing tissues, corresponding well to the GH hypersensitivity demonstrated in obesity.[7,8,25]

IGFBP-1 is regarded as an important inhibitor of IGF-I bioactivity in the circulation.[6,8,11] IGFBP-1 has been demonstrated to be low in compensated insulin resistant states.[8,29] In healthy individuals short-term fasting led to a 50% reduction in free IGF-I concomitantly with an increase in IGFBP-1 and an increase in the complex formation of IGFBP-1 and IGF-I.[30] A decline in free IGF-I level, induced by an increase in IGFBP-1, might serve to protect against possible insulin-like activity of the IGF during fasting.[31] IGFBP-2 has been found to be reduced in insulin-resistant states with high plasma insulin concentrations and to correlate inversely with free IGF-II.[8] In accordance, in the present study, IGFBP-2 correlated inversely with free IGF-II. Interestingly, in the total study population, we observed a significant inverse correlation between fasting plasma insulin concentration versus IGFBP-1 and IGFBP-2 and, in addition, a significant positive correlation between insulin sensitivity and IGFBP-1 and IGFBP-2. Accordingly, the relative lower fasting serum concentrations of IGFBP-1 and IGFBP-2 in insulin-resistant HIV-infected (lipodystrophic) patients might be a physiologic regulator mechanism to provide higher levels of free IGF-I and free IGF-II, which due to their insulin-like effects,[7,32] would support insulin in improving glucose homeostasis. In contrast, the higher IGFBP-1 and IGFBP-2 levels in insulin-sensitive HIV-infected patients (without fat distribution) could be a mechanism to bind an enhanced amount of free IGF-I and free IGF-II to prevent hypoglycemia during fasting.

Table 5. Age-Adjusted Correlations Between log10 IGFBP-1 and log10 IGFBP-2 Versus Insulin Sensitivity, Insulin, and C-Peptide Concentrations

	Log10 IGFBP-1	Log10 IGFBP-2
Log10 $ISI_{composite}$	0.40*	0.72†
Log10 fp-insulin	−0.38*	−0.67†
Log10 fp-C-peptide	−0.40*	−0.64†

NOTE. Analysis were performed for the pooled study groups of 16 LIPO and 13 NONLIPO.

*$P < .05$.

†$P < .001$.

IGFBP-3 is the most abundant IGFBP in the circulation binding more than 75% of the IGF-I and IGF-II.[33] IGFBP-3 is GH-dependent.[10] Therefore, the finding of similar levels of intact IGFBP-3 (as determined by WLB) and total IGFBP-3 (as determined by IRMA) in lipodystrophic HIV-infected patients and HIV-infected patients without fat redistribution supports an increased GH sensitivity of the IGFBP-3 producing tissues in lipodystrophic HIV-infected patients in whom GH secretion likely is impaired.[2,3] In accordance with findings in the healthy background population, IGFBP-3 and IGF-I in the present study were inversely correlated with age,[34] which should be observed when comparing such factors in HIV-infected patients. The fasting concentrations of total and free IGF-I, IGF-II, and IGFBP-3 of the lipodystrophic HIV-infected patients in the present study correspond exactly to the concentrations of such parameters (determined by identical assays) in healthy individuals of similar age and BMI,[7] which strongly support that the levels of GH-sensitive IGFs and IGFBPs in HIV-lipodystrophy are normal.

IGFBP-3 protease is increased in various diseases,[35-37] including insulin resistance and GHD.[13,14] To our knowledge, the present study is the first to measure IGFBP-3 protease in HIV-infected patients. Interestingly, IGFBP-3 protease activity did not differ between the 2 study groups in the present study. In fact, IGFBP-3 protease activity of the 2 study groups was similar to healthy HIV-negative individuals (ie, the control subjects for the assay, see Materials and Methods section) and much lower compared with pregnant individuals (ie, the control subjects for the assay, see Materials and Methods section), a state known to display increased activity of IGFBP-3 protease.[38] The assay of IGFBP-3 protease is depending upon internal controls and should be applied only in a setting where a relevant control group is defined. The suggested function of IGFBP-3 protease is to enhance bioactive IGF-I.[39] Accordingly, we showed after controlling for strong covariate factors of free IGF-I (ie, age, total IGF-I, and IGFBP-3) that IGFBP-3 protease correlated positively with free IGF-I in the total study population and, in particular, in the group of lipodystrophic patients in whom protease activity accounted for approximately 50% of the variability of free IGF-I. Taken together, the inverse correlation between IGFBP-3 and IGFBP-3 protease and the positive correlation between total IGF-I and IGFBP-3 suggest that IGFBP-3 protease activity in lipodystrophic HIV-infected patients may be an important remedy to facilitate relative high bioactive IGF-I in a condition where total IGF-I concentration is relatively low.

It is a limitation of the present study that GH dynamics were not measured. A single fasting GH concentration is a poor surrogate for GH diurnal concentration and secretion pattern due to the highly pulsatile nature of GH secretion[40]; however, the fact that we observed relatively decreased overnight fasting GH concentrations in lipodystrophic HIV-infected patients is consistent with previous observations in lipodystrophic HIV-infected patients showing major impairments in GH secretion as compared with HIV-infected patients without lipodystrophy.[2,3] The relatively increased overnight fasting serum concentration of GHBP in lipodystrophic HIV-infected patients is a novel observation. GHBP does not as GH display diurnal pulsatility, thus fasting concentration reflects, more accurately, mean diurnal concentration.[41] Under normal physiologic conditions, GHBP binds about half of the GH in human serum[42] and acts as a reservoir or a buffer, damping the oscillations of plasma GH, and prolonging GH half-life.[43] It merits research, therefore, to determine whether increased serum GHBP plays a role for the reported aberration in GH pulsatility in HIV-lipodystrophy.[2,3] The close positive association between abdominal fat mass and GHBP levels in our patients is in accordance with our previous findings in HIV-negative healthy subjects.[44] Some evidence suggests that GHBP is a marker of GH receptor responsiveness,[9] and, therefore, the physiologic significance of the increased GHBP concentration in lipodystrophic HIV-infected patients in the present study may be an enhanced GH sensitivity of IGF producing tissues in HIV-lipodystrophy resulting in the observed similar IGF serum concentrations between lipodystrophic and nonlipodystrophic patients despite impaired GH secretion in HIV-lipodystrophy.

HIV-infected patients with wasting displayed GH resistance as demonstrated by a poor increase in IGF-I levels to high-dose GH treatment, ie, after GH therapy, 6 mg/d during 12 weeks, total serum IGF-I concentration of such patients merely was increased by 1-fold to the upper normal limit of IGF-I.[45] In contrast, in HIV-infected patients with fat redistribution, who were treated with GH doses of 2 to 6 mg/d (aiming to reduce intra-abdominal fat mass), total IGF increased by 3- to 6-fold, ie, approximately to levels 2 to 3 times greater than upper normal IGF-I concentration.[46-48] Clearly, IGF-I–producing tissues in lipodystrophic HIV-infected patients appear much more sensitive to injected GH than in HIV-infected patients with wasting. Future studies should address the dose-response relationship between exogenous GH injection and endogenous IGF-I response in lipodystrophic HIV-infected patients to determine the exact GH sensitivity of IGF-I–producing tissues as has recently been examined in healthy HIV negative individuals.[49]

In conclusion, our data suggest that lipodystrophic HIV-infected patients compared with HIV-infected patients without fat redistribution exhibit similar fasting serum concentrations of important GH-sensitive IGFs, IGFBP-3, and IGFBP-3 protease, but exhibit suppression of predominantly insulin-sensitive IGFBPs. Supported by increased fasting GHBP and lower fasting GH concentrations in lipodystrophic HIV-infected patients, our data suggest that such patients display relatively enhanced GH sensitivity of GH target tissues, however this can be determined only by measuring response (eg, IGF-I) to exogenous GH injection. Additionally, our data lend support to the hypothesis that IGFBP-3 protease may be a significant regulator of bioactive IGF-I in HIV-infected patients with lipodystrophy.

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REFERENCES

1. Carr A, Emery S, Law M, et al: An objective case definition of lipodystrophy in HIV-infected adults: A case-control study. Lancet 361:726-735, 2003

2. Koutkia P, Meininger G, Canavan B, et al: Metabolic regulation of growth hormone by free fatty acids, somatostatin, and ghrelin in HIV-lipodystrophy. Am J Physiol 286:E296-E303, 2004

3. Rietschel P, Hadigan C, Corcoran C, et al: Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy. J Clin Endocrinol Metab 86:504-510, 2001

4. Laursen T, Jorgensen JO, Christiansen JS: Metabolic effects of growth hormone administered subcutaneously once or twice daily to growth hormone deficient adults. Clin Endocrinol (Oxf) 41:337-343, 1994

5. Marzullo P, Di Somma C, Pratt KL, et al: Usefulness of different biochemical markers of the insulin-like growth factor (IGF) family in diagnosing growth hormone excess and deficiency in adults. J Clin Endocrinol Metab 86:3001-3008, 2001

6. Skjaerbaek C, Vahl N, Frystyk J, et al: Serum free insulin-like growth factor-I in growth hormone-deficient adults before and after growth hormone replacement. Eur J Endocrinol 137:132-137, 1997

7. Frystyk J, Vestbo E, Skjaerbaek C, et al: Free insulin-like growth factors in human obesity. Metabolism 44:37-44, 1995

8. Frystyk J, Skjaerbaek C, Vestbo E, et al: Circulating levels of free insulin-like growth factors in obese subjects: The impact of type 2 diabetes. Diabetes Metab Rev 15:314-322, 1999

9. Amit T, Youdim MB, Hochberg Z: Clinical review 112: Does serum growth hormone (GH) binding protein reflect human GH receptor function? J Clin Endocrinol Metab 85:927-932, 2000

10. Baxter RC: Insulin-like growth factor binding proteins in the human circulation: A review. Horm Res 42:140-144, 1994

11. Frystyk J, Skjaerbaek C, Dinesen B, et al: Free insulin-like growth factors (IGF-I and IGF-II) in human serum. FEBS Lett 348: 185-191, 1994

12. Skjaerbaek C, Frystyk J, Orskov H, et al: Differential changes in free and total insulin-like growth factor I after major, elective abdominal surgery: The possible role of insulin-like growth factor-binding protein-3 proteolysis. J Clin Endocrinol Metab 83:2445-2449, 1998

13. Bang P, Brismar K, Rosenfeld RG: Increased proteolysis of insulin-like growth factor-binding protein-3 (IGFBP-3) in noninsulin-dependent diabetes mellitus serum, with elevation of a 29-kilodalton (kDa) glycosylated IGFBP-3 fragment contained in the approximately 130- to 150-kDa ternary complex. J Clin Endocrinol Metab 78:1119-1127, 1994

14. Lassarre C, Lalou C, Perin L, et al: Protease-induced alteration of insulin-like growth factor binding protein-3 as detected by radioimmunoassay: Agreement with ligand blotting data. Growth Regul 4:48-55, 1994

15. Puavilai G, Chanprasertyotin S, Sriphrapradaeng A: Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. World Health Organization. Diabetes Res Clin Pract 44:21-26, 1999

16. World Health Organization: Diabetes Mellitus: Report of a WHO Study Group. Geneva World Health Org, Tech Rep Ser, no.727, 1985

17. Frystyk J, Dinesen B, Orskov H: Non-competitive time-resolved immunofluorometric assays for determination of human insulin-like growth factor I and II. Growth Regul 5:169-176, 1995

18. Hossenlopp P, Seurin D, Segovia-Quinson B, et al: Analysis of serum insulin-like growth factor binding proteins using western blotting: Use of the method for titration of the binding proteins and competitive binding studies. Anal Biochem 154:138-143, 1986

19. Flyvbjerg A, Kessler U, Dorka B, et al: Transient increase in renal insulin-like growth factor binding proteins during initial kidney hypertrophy in experimental diabetes in rats. Diabetologia 35:589-593, 1992

20. Lamson G, Giudice LC, Rosenfeld RG: A simple assay for proteolysis of IGFBP-3. J Clin Endocrinol Metab 72:1391-1393, 1991

21. Fisker S, Frystyk J, Skriver L, et al: A simple, rapid immunometric assay for determination of functional and growth hormone-occupied growth hormone-binding protein in human serum. Eur J Clin Invest 26:779-785, 1996

22. Matsuda M, DeFronzo RA: Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. Diabetes Care 22:1462-1470, 1999

23. Monzavi R, Cohen P: IGFs and IGFBPs: Role in health and disease. Best Pract Res Clin Endocrinol Metab 16:433-447, 2002

24. Collett-Solberg PF, Cohen P: Genetics, chemistry, and function of the IGF/IGFBP system. Endocrine 12:121-136, 2000

25. Hochberg Z, Hertz P, Colin V, et al: The distal axis of growth hormone (GH) in nutritional disorders: GH-binding protein, insulin-like growth factor-I (IGF-I), and IGF-I receptors in obesity and anorexia nervosa. Metabolism 41:106-112, 1992

26. Grinspoon S, Corcoran C, Stanley T, et al: Effects of androgen administration on the growth hormone-insulin-like growth factor I axis in men with acquired immunodeficiency syndrome wasting. J Clin Endocrinol Metab 83:4251-4256, 1998

27. Mulligan K, Grunfeld C, Hellerstein MK, et al: Anabolic effects of recombinant human growth hormone in patients with wasting associated with human immunodeficiency virus infection. J Clin Endocrinol Metab 77:956-962, 1993

28. Helle SI, Ueland T, Ekse D, et al: The insulin-like growth factor system in human immunodeficiency virus infection: Relations to immunological parameters, disease progression, and antiretroviral therapy. J Clin Endocrinol Metab 86:227-233, 2001

29. Nam SY, Lee EJ, Kim KR, et al: Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. Int J Obes Relat Metab Disord 21:355-359, 1997

30. Norrelund H, Frystyk J, Jorgensen JO, et al: The effect of growth hormone on the insulin-like growth factor system during fasting. J Clin Endocrinol Metab 88:3292-3298, 2003

31. Katz LE, DeLeon DD, Zhao H, et al: Free and total insulin-like growth factor (IGF)-I levels decline during fasting: Relationships with insulin and IGF-binding protein-1. J Clin Endocrinol Metab 87:2978-2983, 2002

32. Guler HP, Zapf J, Froesch ER: Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. N Engl J Med 317:137-140, 1987

33. Jones JI, Clemmons DR: Insulin-like growth factors and their binding proteins: Biological actions. Endocr Rev 16:3-34, 1995

34. Gomez JM, Maravall FJ, Gomez N, et al: Interactions between serum leptin, the insulin-like growth factor-I system, and sex, age, anthropometric and body composition variables in a healthy population randomly selected. Clin Endocrinol (Oxf) 58:213-219, 2003

35. Bereket A, Lang CH, Blethen SL, et al: Insulin-like growth factor binding protein-3 proteolysis in children with insulin-dependent diabetes mellitus: A possible role for insulin in the regulation of IGFBP-3 protease activity. J Clin Endocrinol Metab 80:2282-2288, 1995

36. Cotterill AM, Holly JM, Taylor AM, et al: The insulin-like growth factor (IGF)-binding proteins and IGF bioactivity in Laron-type dwarfism. J Clin Endocrinol Metab 74:56-63, 1992

37. Timmins AC, Cotterill AM, Hughes SC, et al: Critical illness is associated with low circulating concentrations of insulin-like growth factors-I and -II, alterations in insulin-like growth factor binding proteins, and induction of an insulin-like growth factor binding protein 3 protease. Crit Care Med 24:1460-1466, 1996

38. Giudice LC, Farrell EM, Pham H, et al: Insulin-like growth factor binding proteins in maternal serum throughout gestation and in the puerperium: Effects of a pregnancy-associated serum protease activity. J Clin Endocrinol Metab 71:806-816, 1990

39. Bunn RC, Fowlkes JL: Insulin-like growth factor binding protein proteolysis. Trends Endocrinol Metab 14:176-181, 2003

40. Heijligenberg R, Sauerwein HP, Brabant G, et al: Circadian growth hormone secretion in asymptomatic human immune deficiency virus infection and acquired immunodeficiency syndrome. J Clin Endocrinol Metab 81:4028-4032, 1996

41. Ho PJ, Gesundheit N, Wong WL, et al: Dynamic changes of growth hormone-binding protein concentrations in normal men and patients with acromegaly: Effects of short-term fasting. Metabolism 44:667-672, 1995

42. Baumann G, Amburn K, Shaw MA: The circulating growth hormone (GH)-binding protein complex: A major constituent of plasma GH in man. Endocrinology 122:976-984, 1988

43. Barnard R, Waters MJ: The serum growth hormone binding protein: Pregnant with possibilities. J Endocrinol 153:1-14, 1997

44. Fisker S, Vahl N, Jorgensen JO, et al: Abdominal fat determines growth hormone-binding protein levels in healthy nonobese adults. J Clin Endocrinol Metab 82:123-128, 1997

45. Schambelan M, Mulligan K, Grunfeld C, et al: Recombinant human growth hormone in patients with HIV-associated wasting: A randomized, placebo-controlled trial. Serostim Study Group. Ann Intern Med 125:873-882, 1996

46. Engelson ES, Glesby MJ, Mendez D, et al: Effect of recombinant human growth hormone in the treatment of visceral fat accumulation in HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 30:379-391, 2002

47. Kotler DP, Muurahainen N, Grunfeld C, et al: Effects of growth hormone on abnormal visceral adipose tissue accumulation and dyslipidemia in HIV-infected patients. J Acquir Immune Defic Syndr Hum Retrovirol 35:239-252, 2004

48. Lo JC, Mulligan K, Noor MA, et al: The effects of recombinant human growth hormone on body composition and glucose metabolism in HIV-infected patients with fat accumulation. J Clin Endocrinol Metab 86:3480-3487, 2001

49. Ghigo E, Aimaretti G, Maccario M, et al: Dose-response study of GH effects on circulating IGF-I and IGFBP-3 levels in healthy young men and women. Am J Physiol 276:E1009-E1013, 1999