CLINICAL STUDY

GH response to GHRH plus arginine is impaired in lipotrophic women with human immunodeficiency virus compared with controls

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Abstract

Objective: GH secretion is impaired in lipodystrophic human immunodeficiency virus (HIV) patients and inversely related to lipodystrophy-related fat redistribution in men. Less is known about the underlying mechanisms involved in reduced GH secretion in HIV-infected women.

Design: A case–control, cross-sectional study comparing GH/IGF1 status, body composition, and metabolic parameters in 92 nonobese women with HIV-related lipodystrophy and 63 healthy controls matched for age, ethnicity, sex, and body mass index (BMI).

Methods: GH, IGF1, IGF binding protein 3 (IGFBP3), GH after GHRH plus arginine (GHRH + Arg), several metabolic variables, and body composition were evaluated.

Results: GH response to GHRH + Arg was lower in HIV-infected females than in controls. Using a cutoff of peak GH ≤ 7.5 $\mu\text{g/l}$, 20.6% of HIV-infected females demonstrated reduced peak GH response after GHRH + Arg. In contrast, none of the control subjects demonstrated a peak GH response ≤ 7.5 $\mu\text{g/l}$. Bone mineral density (BMD), quality of life, IGF1, and IGFBP3 were lowest in the HIV-infected females with a GH peak ≤ 7.5 $\mu\text{g/l}$. BMI was the main predictive factor of GH peak in stepwise multiregression analysis followed by age, with a less significant effect of visceral fat in the HIV-infected females.

Conclusions: This study establishes that i) GH response to GHRH + Arg is lower in lipotrophic HIV-infected women than in healthy matched controls, ii) BMI more than visceral adipose tissue or trunk fat influences GH peak in this population, and iii) HIV-infected women with a GH peak below or equal to 7.5 $\mu\text{g/l}$ demonstrate reduced IGF1, IGFBP3, BMD, and quality of life.

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Introduction

Human immunodeficiency virus 1 (HIV-1)-infected patients undergoing treatment with highly active antiretroviral therapy (HAART) demonstrate a number of endocrine and metabolic complications (1, 2). Reduced GH secretion occurs in about one-third of HIV-infected male patients (3, 4) with HIV/HAART-associated lipodystrophy (HAL) (5). GH response to GHRH plus arginine (GHRH + Arg) is impaired in HIV-infected men compared with age- and body mass index (BMI)-matched male HIV-uninfected controls (6, 7, 8). In HIV-infected men, however, reduced GH secretion occurs more frequently in patients with lipodystrophy (6, 9), while no study compared lipodystrophic with nonlipodystrophic women. Accordingly, the GH/IGF1 axis has been less well studied in HIV-infected women (3, 7) compared with men (6, 7, 8, 9). Among females, GH secretion did not differ between HIV and control subjects (3, 7), while no data are available in relatively

lipotrophic HIV-infected women. Reduced GH secretion is related to fat redistribution (3, 4, 6, 7, 8, 9) and elevated free fatty acids (FFA) in men (3, 4, 9), but this association seems to be less pronounced or absent in women (3, 7). Among HIV-infected (3, 7) and HIV-uninfected subjects (10), gender differences in GH secretion and body composition effects are seen (11, 12).

The GHRH + Arg test has been used as a tool for diagnosing adult-onset GH deficiency (GHD) (13, 14, 15, 16). GHRH + Arg, however, is less accurate in overweight and obese patients due to the inhibition of GH secretion exerted by adipose tissue, especially visceral fat (10, 17). To improve GHRH + Arg reliability and prevent misdiagnosis, more restrictive, BMI-based cutoffs are preferred in obese and overweight HIV-uninfected subjects (14, 18). The increase in FFA, a phenomenon related to visceral obesity, may explain the blunted GH secretion in HIV-infected men (9). However, other mechanisms are possible (9, 19). HIV-infected

patients demonstrate significant changes in body composition (5) and may thus demonstrate abnormalities in GH secretion. Moreover, consideration has been given to increasing GH in HIV patients to improve metabolic abnormalities, and recombinant human GH (r-hGH) as well as a GHRH-releasing analog, recently been approved by the FDA in this regard, have been considered (6, 7, 20, 21, 22, 23).

In this case-control, cross-sectional study, we compared the GH response to GHRH + Arg in lipodystrophic, nonobese, HIV-infected women with HAL and healthy HIV-uninfected controls matched for sex, age, ethnicity, and BMI. In order to avoid the possible effect of obesity on GH secretion (10, 14, 17, 18), we chose to enroll nonobese HIV-infected females. The aim of the study was to investigate the prevalence of GHD in these HIV-infected females and explore the relationship among GH/IGF1 status, body composition, and metabolic parameters.

Subjects and methods

Subjects

We performed a case-control, cross-sectional study at the University of Modena and Reggio Emilia by prospectively enrolling 92 Caucasian female outpatients with HIV-related HAL and 63 healthy control women. The HIV-infected female patients were consecutively selected according to inclusion and exclusion criteria among female outpatients attending the Clinic of Infectious Diseases over a period from October 2005 to December 2010. Similarly, healthy control women were selected among asymptomatic women with documented normal thyroid hormones who referred to the Laboratory and Chair of Endocrinology of the University of Modena and Reggio Emilia from January 2006 to December 2010 for a screening program of thyroid function. The patients and the controls were matched for sex, ethnicity, age – between 18 and 57 years – and BMI with a difference minimization approach. In order to avoid obesity as a confounding factor for the study of GH secretion, we restricted the enrollment to nonobese HIV-infected and -uninfected females with a BMI ≤ 29.9 kg/m². With this criterion of enrollment, the HIV-infected patients demonstrated relative lipodystrophy in terms of changes in body composition (see Results section for details). Basal anthropometric and biochemical evaluations are summarized in Table 1.

Inclusion criteria The HIV-infected women had a physician-confirmed diagnosis of lipodystrophy according to the HIV Outpatients Study definition (24), with or without metabolic alterations; they had documented HIV infection for more than 5 years and were receiving HAART for least 6 months.

Exclusion criteria The exclusion criteria were: a BMI ≥ 30 kg/m²; diabetes mellitus; signs, symptoms, or a medical history of pituitary disease; a previous conventional pituitary surgery or radiotherapy; hypogonadism; pregnancy; previous or current treatment with corticosteroids and/or exogenous estrogens; use of oral hypoglycemic drugs, insulin; and a history of r-hGH treatment within the 3 months preceding enrollment in the study.

Methods

All subjects underwent venous blood sampling at 0800 h after an overnight fast. All blood samples were stored at -80°C until assayed in the same laboratory. The population enrolled for the research protocol underwent biochemical and endocrinological evaluations including GHRH + Arg testing.

Assessment of GH secretion The GH-IGF1 system was studied under basal condition assaying serum GH, IGF1, and insulin growth factor binding protein 3 (IGFBP3) and under dynamic testing.

A standardized GHRH + Arg test was performed (GHRH 1–29; GEREFF, Serono; 1 $\mu\text{g/kg}$ i.v. at 0 min; arginine chloride, 0.5 g/kg i.v. for 30 min, from 0 to +30 min, up to a dose of 30 g). Blood samples were obtained every 15 min from -15 to $+60$ min and then every 30 min till 120 min, as previously standardized (15, 18). GH peak response to GHRH + Arg test was evaluated in all the 155 subjects enrolled in the study according to different cutoff values (6, 7, 15, 16, 18). The threshold of GH peak of 7.5 $\mu\text{g/l}$ was used to separate normal from impaired responses to GHRH + Arg in both the HIV-infected patients and the healthy controls as it was previously used in studies on HIV-infected subjects (6, 7, 8) and it is close to that of 8.0 $\mu\text{g/l}$, which is commonly used for overweight subjects (18). The parameters used to evaluate the GH response to GHRH + Arg were the serum GH peak and the area under the curve (AUC).

Other hormonal and biochemical parameters

Serum estradiol, glucose, insulin, and lipid profile (triglycerides, total cholesterol, LDL, and HDL) were measured in all subjects.

HIV infection-related parameters The HIV infection characteristics (i.e. duration of HIV infection, Centers for Disease Control and Prevention classification, antiretroviral class and single-drug exposure, and Nadir CD4 count) were obtained from medical records. The CD4 cell count and quantitative plasma HIV RNA viral load were assayed in the 92 HIV-infected patients.

Body composition and adipose tissue distribution

Demographic characteristics (age, race, and sex) and anthropometric measurements were obtained during the medical examination and by means of dual-energy X-ray absorptiometry (DXA) for all the subjects enrolled,

Table 1 Baseline demographic, anthropometric, HIV-related parameters, biochemical and hormonal data of HIV-infected women with highly active antiretroviral therapy (HAART)-associated lipodystrophy (HAL) and of HIV-uninfected healthy females. Values are expressed as median (min-max).

	Normal range	HIV-infected patients (n=92) Median (min-max)	HIV-uninfected control subjects (n=63) Median (min-max)	P value
Age (years)	NA	43.5 (18-56)	44 (18-57)	0.67
Body composition				
Weight (kg)	NA	60.2 (48-90)	66 (42-80.5)	0.03
BMI (kg/m ²)	<30	24.2 (18.5-29.9)	24.6 (16.4-29.9)	0.5
WHR	<0.80	0.9 (0.8-1.1)	0.8 (0.6-1.3)	<0.0001
Waist circumference (cm)	<80	87.5 (70-122)	80 (58-111)	<0.0001
SAT at CT scan (cm ²)	NA	198 (33-590)	NA	NA
VAT at CT scan (cm ²)	NA	107 (34-304)	NA	NA
TAT at CT scan (cm ²)	NA	334.5 (82-776)	NA	NA
VAT/SAT at CT scan	NA	0.5 (0.1-2.7)	NA	NA
DXA total fat mass (g)	NA	15 577 (7106-35 645)	21 684 (8245-44 351)	<0.0001
DXA total lean mass (g)	NA	41 834 (26 921-59 152)	42 278 (26 366-51 181)	0.2
DXA trunk fat mass (g)	NA	8749 (3855-17 471)	7849 (2980-19 931)	0.2
DXA trunk lean mass (g)	NA	21 274 (13 027-31 753)	21 302.9 (12 553-25 649.9)	0.01
DXA limbs fat mass (g)	NA	3116.5 (810-9495)	9080 (3760-13 839.7)	<0.0001
DXA limbs lean mass (g)	NA	13 058 (9072-20 329)	13 032.1 (4208.4-15 717.3)	0.9
Hormonal and biochemical data				
Serum IGF1 (nmol/l [ng/ml])	12.31-34.97 [94-267]	18.3 (3.2-51.7) [140.5 (25-395)]	20 (9-31.9) [153 (69.4-244)]	0.8
Serum IGFBP3 (nmol/l [ng/ml])	122.40-248.40 [3400-6900]	123.7 (18-265.3) [3436.5 (500-7370)]	139.2 (66.7-182.1) [3869 (1854-5061)]	0.07
GH peak after GHRH + Arg (μg/l [ng/ml])	> 9 [> 9]	18.5 (1.5-80) [18.5 (1.5-80)]	39.5 (8.6-89) [39.5 (8.6-89)]	<0.0001
AUC	NA	1151.5 (112.5-6126.4)	2994.7 (366-7674.7)	<0.0001
Serum glucose (mmol/l [mg/dl])	3.88-6.10 [70-110]	4.99 (3.9-7) [90 (71-127)]	4.55 (3.3-5.8) [82 (60-105)]	<0.0001
Serum insulin (pmol/l [μIU/ml])	<107.62 [<15]	110.4 (5-546.7) [15.4 (0.7-76.2)]	50.9 (17.9-144.2) [7.1 (2.5-20.1)]	<0.0001
Serum triglycerides (mmol/l [mg/dl])	0.45-1.69 [40-150]	1.4 (0.45-8.9) [127.5 (40-790)]	0.9 (0.4-2) [80 (38-178)]	<0.0001
Serum estradiol (pmol/l [pg/ml])	73.42-1468.4 [20-400]	304.6 (36.71-2573.3) [83 (10-701)]	375.9 (73.42-2298) [102.4 (20-626)]	0.08
HIV-related parameters				
CD4 count (cells/mm ³)	> 800	546.28 ± 271.92	NA	NA
Viral load, log (copies/ml)	Undetectable	2.24 ± 0.97	NA	NA
Antiretroviral therapy (HAART)				
PI therapy (%)	NA	91	NA	NA
NRTI therapy (%)	NA	100	NA	NA
NNRTI therapy (%)	NA	58	NA	NA

PI, protease inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; NA, not applicable. Values expressed in conventional units appear in square brackets.

while an abdominal CT scan was performed only on the 92 HIV-infected patients. The healthy controls did not receive a CT scan for ethical reasons (see Ethics section).

We measured weight after a 5-h fast and height by stadiometer; we calculated waist and hip circumferences as the average of three measurements.

A whole body composition study (lean and fat mass) was performed by DXA (Hologic, Inc., Waltham, MA, USA) according to standardized procedures (25) and with precision of 3% for the measurement of fat mass and 1.5% for fat-free mass (26). The abdominal adipose tissue distribution was studied by CT scans at L4 level, with a single-slice abdominal scan as in standard protocols. Abdominal visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) were determined (27), total abdominal adipose tissue (TAT) being the sum of VAT and SAT.

Assessment of quality of life In a subset of 70 HIV-infected patients and 49 control women, we evaluated quality of life by the two validated questionnaires for GHD in adults: the Questions on Life Satisfaction Hypopituitarism (QLS-H) and Adult GH Deficiency Assessment (AGHDA) (28). The scores were calculated according to standard instructions (28).

Laboratory analysis Serum IGF1 was measured by RIA (INCSTAR Corp., Stillwater, MN, USA), with linear reading up to 0.002 nmol/l and inter- and intra-assay coefficients of variation of 12 and 8% respectively. Serum IGFBP3 was measured by immunochemiluminescence (LIAISON Nichols Advantage, San Juan Capistrano, CA, USA), with inter- and intra-assay coefficients of variation of 4.8 and 5% respectively. Serum GH was measured using a fluoroimmuno-metric assay (LIAISON DIASORIN Autodelphia hGH kit, Stillwater, MN, USA) with a sensitivity of 0.02 µg/l, linear reading up to 80 µg/l, and inter- and intra-assay coefficients of variation of 5.5 and 4.9% respectively. Serum estradiol was assayed by RIA (Third-Generation DSL-39100; Diagnostic Systems Laboratories, Inc., Webster, TX, USA) with a sensitivity of 2.2 pmol/l (0.6 pg/ml), and the coefficients of variation were 4.1–9.9% (inter-assay) and 3.4–3.9% (intra-assay). Glucose, insulin, and lipid profile (triglycerides, total cholesterol, LDL, and HDL) were assayed using commercially available kits. The CD4 cell count was performed by flow cytometry (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Quantitative plasma HIV RNA viral load was performed by ultrasensitive method (Amplicor HIV-1 Monitor Assay; Roche Molecular Systems), with the detection range between 50 and 75 000 copies/ml.

Statistical analysis

The nonparametric Mann–Whitney *U* test was used for comparisons because most of the variables were not

normally distributed by the Kolmogorov–Smirnov test. The comparison among groups was performed using the nonparametric Kruskal–Wallis test (when more than two groups) followed by the Dunn's multiple comparison *post hoc* test when a significant difference was found in order to determine differences between individual groups. All the data are shown as median and minimum to maximum (min–max) in parenthesis.

The number of subjects with normal or impaired GH peak responses to GHRH + Arg according to different cutoffs is expressed as percentages.

We evaluated demographic, anthropometric, metabolic, and hormonal variables in order to identify possible predictive factors for GH response to GHRH + Arg in HIV-infected females. A stepwise, linear, multiple regression analysis was performed using serum GH peak and the AUC as dependent variables in two different analyses, and age, BMI, total lean body mass, trunk lean mass, trunk fat mass, insulin, triglycerides, and waist circumference as independent variables. A second, stepwise, linear, multiple regression analysis was performed only in HIV-infected individuals by using independent variables of body composition obtained by abdominal CT scan (VAT, SAT, and TAT) instead of those obtained by DXA, without modifying the dependent variables and all the other independent variables.

All multiple regression analyses were based on a single regression analysis for each predictor independent variable that allowed identifying candidate predictive variables. A univariate analysis was performed before multivariate analysis (Supplementary Tables 1 and 2, see section on supplementary data given at the end of this article). The independent variables with significance of *P* value <0.05 were included in the regression model. Stepwise, linear, multiple regression analysis using a backward elimination method was applied to the data with *P* <0.10 as the criterion for a variable to be considered in the model. The percentage contribution of a given variable to the variance of both the GH peak and the AUC was determined by using the square of the Pearson correlation coefficient (r^2).

Statistical analysis was performed using the 'Statistical Package for the Social Sciences' software for Windows (version 16.0; SPSS, Inc., Chicago, IL, USA) and Sigma Plot (version 11.00 for Windows; Systat Software, Inc., San Jose, CA, USA) for Kruskal–Wallis and the Dunn's tests. For all comparisons, *P* values <0.05 were considered statistically significant.

Ethics

All subjects (both HIV-infected patients and HIV-uninfected healthy females) gave written informed consent for participation in the study and for the publication of the data. The local Institutional Review Board (Comitato Etico di Modena) approved the study (N. 158/07). The 63 healthy controls did not receive

a CT scan to avoid any radiation exposure. HIV-infected females underwent CT scanning of the abdomen to obtain information on the relationship between fat accumulation and GH secretion.

Results

Ninety-two nonobese HIV-infected lipodystrophic female patients were compared with 63 HIV-negative matched controls (Table 1).

Weight, total fat mass, trunk lean mass, and limb fat mass at DXA were significantly lower in the HIV-infected females than in uninfected females (Table 1), but BMI was similar. Waist circumference and WHR values were significantly higher in HIV-infected females than in controls ($P < 0.0001$). The percentage of total body fat mass was lower than in controls (27 vs 34%). HIV-infected females did not show any statistically significant difference in other variables of body composition when compared with controls. Serum glucose, insulin, and triglycerides were significantly higher in the HIV-infected females than in controls ($P < 0.0001$; Table 1). GH peak and AUC were significantly lower in HIV-infected females than in controls ($P < 0.0001$), while the IGF1 and IGFBP3 levels did not significantly differ between the two groups (Table 1). Of the 92 HIV-infected women, ten (10.9%) were postmenopausal; similarly, seven of the 63 HIV-uninfected women (11.0%) were postmenopausal. Thus, the percentage of pre- and postmenopausal women did not differ between HIV-infected patients and controls. Of the postmenopausal women, four of ten HIV-infected women had a GH peak below 9 $\mu\text{g/l}$, while none of the seven HIV-uninfected postmenopausal women had a GH peak below 9 $\mu\text{g/l}$.

When different cutoffs for the GH peak after GHRH + Arg were considered, the percentage of HIV-infected patients failing to reach a normal GH peak varied according to the cutoff used (Fig. 1). Using restrictive cutoffs such as 4.2 and 5 $\mu\text{g/l}$, 8.7% ($n = 8$ subjects) of the HIV-infected patients failed to reach a GH peak above both these thresholds (Fig. 1). Using the cutoffs of 7.5 and 8 $\mu\text{g/l}$, 20.6 and 22.8%, respectively ($n = 19$ and 21 subjects respectively) of the HIV-infected patients failed to reach a GH peak above these thresholds (Fig. 1). Finally, using a cutoff of 9 $\mu\text{g/l}$, commonly used in clinical practice for normal, healthy nonobese subjects, the percentage of HIV-infected patients with reduced GH response rose to 28.2% ($n = 26$ subjects; Fig. 1). Among the 63 controls, only one (1.6%) failed to reach a GH peak above 9 $\mu\text{g/l}$ (GH peak of 8.6 $\mu\text{g/l}$; Fig. 1).

When the HIV-infected females were divided according to the cutoff of 7.5 $\mu\text{g/l}$, also used previously in the context of HIV infection (6, 7, 8), IGF1 and IGFBP3, lumbar, femoral, and total body bone mineral density (BMD) were significantly lower in the HIV-infected

Figure 1 Comparison of GH peak responses between HIV-infected patients and HIV-uninfected healthy controls based on the different cutoffs considered.

women with a GH peak ≤ 7.5 $\mu\text{g/l}$ than in HIV-infected females with a GH peak > 7.5 $\mu\text{g/l}$ ($P < 0.001$; Supplementary Table 3, see section on supplementary data given at the end of this article). Of interest, in a subset of participants, the quality-of-life scores were lower in the 15 HIV-infected women with a GH peak ≤ 7.5 $\mu\text{g/l}$ than in 55 HIV-infected females with a GH peak > 7.5 $\mu\text{g/l}$ ($P < 0.001$; Supplementary Table 3).

As far as body composition parameters at CT scan are concerned, SAT, VAT, and TAT did not differ significantly between the HIV-infected women with a GH peak ≤ 7.5 $\mu\text{g/l}$ (SAT: median 184 cm^2 , VAT: median 142 cm^2 , and TAT: median 362 cm^2) and the HIV-infected women with a GH peak > 7.5 $\mu\text{g/l}$ (SAT: median 207 cm^2 , VAT: median 104 cm^2 , and TAT: median 325 cm^2).

Estradiol in univariate analysis was not associated with GH peak and AUC in either HIV-infected women (Supplementary Table 1) or controls (Supplementary Table 2). BMI, age, total lean mass, trunk lean mass, trunk fat mass, waist circumference, VAT, and TAT were associated with both GH peak and AUC in HIV-infected women (Supplementary Table 1). BMI, age, trunk fat mass, insulin, triglycerides, and waist circumference were all associated with both GH peak and AUC in

controls (Supplementary Table 2). When considering CT-scan parameters, GH peak ($r=0.318$) and AUC ($r=0.319$) were inversely related to VAT. Stepwise, linear, multiple regression analysis of the HIV-infected group, using the parameters of body fat measured by DXA as independent variables, indicated that BMI was the most significant predictive factor for GH peak after GHRH + Arg ($r^2=0.11$), followed by age and total lean mass when the GH peak was the dependent variable (Table 2), while BMI was the most significant predictive factor for AUC ($r^2=0.15$), followed by age when AUC was the dependent variable (Table 2).

Stepwise, linear, multiple regression analysis of the HIV-infected group, using the parameters of body fat measured by abdominal CT scan (SAT, VAT, and TAT) instead of those obtained by DXA as independent variables, indicated that BMI was the most significant predicting factor of both GH peak after GHRH + Arg ($r^2=0.11$) and AUC ($r^2=0.15$) followed by age (Table 2).

When HIV status was added into a combined regression model, a clear status effect was demonstrated, with HIV infection being the most important predictor of both low GH peak (β -coefficient: 13.315, P level <0.0001) and AUC (β -coefficient: 1089.645, P level <0.0001).

Discussion

To our knowledge, this is the largest study of HIV-infected women investigating GH secretion in comparison with healthy matched controls. This study establishes that i) GH response to GHRH + Arg is lower in HIV-infected women with lipoatrophy than in healthy matched controls, ii) GH peak after GHRH + Arg is impaired in $\sim 20\%$ of HIV-infected women with lipoatrophy depending on the cutoff used, iii) BMI more than VAT or trunk fat influences GH peak among lipoatrophic HIV-infected women, and iv) HIV-infected women with a GH peak ≤ 7.5 $\mu\text{g/l}$ demonstrate reduced serum IGF1, IGFBP3, BMD, and quality of life and may thus be considered relatively GH deficient.

In this large sample of subjects, both the GH peak and the AUC after GHRH + Arg were significantly lower in HIV-infected women with lipoatrophy than in healthy controls, thus demonstrating that pituitary GH reserve may be impaired in HIV-infected women, as previously suggested in men (6, 7, 8, 9). Different from previous studies (7, 8), GH secretion was significantly different in HIV-infected women compared with controls. Even though it is difficult to compare results coming from different settings, the GH peak in these HIV-infected women seems to be lower, on average, with respect to that obtained by Koutkia *et al.* (7). The percentage of postmenopausal women is similar in HIV-infected patients and controls and serum estradiol levels did not influence GH peak and AUC (Supplementary Tables 1 and 2).

However, as HIV-infected patients are mainly lipoatrophic, in this study they represent a different patient population from females of approximately the same age in previous studies (7) and caution is therefore needed in drawing conclusions when comparing different studies due to the GH assay variability (13, 14) and other factors, such as ethnicity (29), which could lead to different results. Since GHRH + Arg only tests pituitary reserve, this methodological approach does not allow us to obtain information about hypothalamic functioning in the current study.

In this paper, a significant percentage of HIV-infected women with lipoatrophy had an impaired GH peak after GHRH + Arg according to the various cutoffs used for both general (15, 16) and/or overweight-obese populations (18). The percentage of HIV-infected patients failing to respond to GHRH + Arg varied from 28.2 to 20.6%, and 8.7% when the cutoffs of 9.0, 7.5, and 4.2 $\mu\text{g/l}$ respectively were considered (Fig. 1). Although relatively modest, these percentages are of significance when compared with those obtained in healthy controls (15, 18) or epidemiological data on the prevalence of GHD in adults (30, 31, 32). At present, the most appropriate cutoffs to define deficient GH secretory response to GHRH + Arg in the context of HIV remain unknown.

The strength of this study lies in the direct measurement of fat tissue distribution by DXA in both patients and controls. DXA, even though less accurate than CT scan, is a valid tool for estimating truncal adiposity, being a valid surrogate and strongly representative of VAT (26). In the univariate analyses (Supplementary Table 1), GH response to GHRH + Arg, in terms of GH peak and AUC, was related to truncal fat in HIV-infected and -uninfected subjects, but this association was not seen in the multivariate analysis. When considering CT scan parameters, GH peak and AUC were inversely related to VAT in univariate analysis but not in multivariate regression analysis among the HIV-infected patients in whom VAT was assessed by CT (Supplementary Table 1).

Taken together, our findings suggest that HIV status was the strongest predictor of both low GH peak and AUC among all the subjects. Visceral adiposity was inversely related to GH response to GHRH + Arg, but its effect was less than expected and is less than that exerted by BMI. Possible explanations for the stronger influence of BMI than VAT on GH peak and AUC, in this study, could be that HIV-infected patients were primarily lipoatrophic and obese subjects were excluded. Taken together, these findings suggest that factors in addition to excess visceral adiposity help to explain reduced GH secretion in HIV-infected women. Among them, the HIV itself, HAART treatments, the poor general health in these patients, their chronic inflammation, and alterations in immune system are all factors that need to be further investigated since they may act centrally on the hypothalamic-pituitary unit to modify pituitary

Table 2 (A) Stepwise regression models estimating predictive factors for GH peak response to GHRH + Arg in the HIV-infected patients by considering as independent variables adiposity measured by both dual-energy X-ray absorptiometry (DXA) or abdominal CT scan. (B) Stepwise regression models estimating predictive factors for AUC of the GH response to GHRH + Arg in the HIV-infected patients by considering as independent variables adiposity measured by both DXA or abdominal CT scan.

	Model 1			Model 2			Model 3		
	β	P	r ²	β	P	r ²	β	P	r ²
(A) GH peak-dependent variable									
By DXA method									
Variables in the model									
BMI	-1.767	0.001	0.11	-1.744	0.001	0.16	-1.208	0.001	0.196
Age				-0.485	0.038		-0.515	0.038	
Total lean body mass							0.001	0.048	
Variables not in the model									
Model 1									
Age, total lean body mass at DXA, trunk lean mass at DXA, trunk fat mass at DXA, insulin, triglycerides, and waist circumference									
Model 2									
Total lean body mass at DXA, trunk lean mass at DXA, trunk fat mass at DXA, insulin, triglycerides, and waist circumference									
Model 3									
Trunk lean mass at DXA, trunk fat mass at DXA, insulin, triglycerides, and waist circumference									
By CT scan									
Variables in the model									
BMI	-1.767	0.001	0.11	-1.744	0.001	0.16			
Age				-0.485	0.038				
Variables not in the model									
Model 1									
Age, insulin, triglycerides, waist circumference, and TAT, SAT, and VAT									
Model 2									
Insulin, triglycerides, waist circumference, and TAT, SAT, and VAT									
(B) AUC-dependent variable									
By DXA method									
Variables in the model									
BMI	-151.94	0.0001	0.15	-150.229	0.0001	0.19			
Age				-35.441	0.042				
Total lean body mass									
Variables not in the model									
Model 1									
Age, total lean body mass at DXA, trunk lean mass at DXA, trunk fat mass at DXA, insulin, triglycerides, and waist circumference									
Model 2									
Total lean body mass at DXA, trunk lean mass at DXA, trunk fat mass at DXA, insulin, triglycerides, and waist circumference									
Model 3									
Trunk lean mass at DXA, trunk fat mass at DXA, insulin, triglycerides, and waist circumference									
By CT scan									
Variables in the model									
BMI	-151.95	0.0001	0.15	-150.23	0.0001	0.19			
Age				-35.45	0.042				
Variables not in the model									
Model 1									
Age, insulin, triglycerides, waist circumference, and TAT, SAT, and VAT									
Model 2									
Insulin, triglycerides, waist circumference, and TAT, SAT, and VAT									

β , Coefficient of regression; SE, standard error of β ; t , t value of β . Sig., significance of two-tailed test.

hormonal secretion (19). Recently, we demonstrated a high rate of secondary hypogonadism (low testosterone and low to normal LH) in a large cohort of HIV-infected men (33), suggesting that the function of the hypothalamic–pituitary unit could be impaired in HIV infection. Since the design of this study was not specifically addressed at identifying the underlying mechanisms involved in the impaired GH secretion, we may only speculate on possible hypothalamic–pituitary dysfunction in HIV infection.

In addition to reduced serum levels of IGF1 and IGFBP3, HIV-infected females with peak GH ≤ 7.5 $\mu\text{g/l}$ showed reduced quality of life and impaired bone health in comparison with HIV-infected patients with a normal GH peak (Supplementary Table 3). These findings support the idea that HIV-infected females with impaired GH peak demonstrate some of the features of GHD associated with traditional hypopituitarism (34, 35). Although unlikely, we cannot exclude hypothalamic–pituitary diseases because the morphology of this area was not studied by MRI. In the absence of confirmed abnormalities of the hypothalamic–pituitary unit, a diagnosis of true GHD could not be confirmed in these HIV-infected individuals because provocative testing with GHRH+Arg must be put into the appropriate clinical context of a known pituitary or hypothalamic process (13, 14, 35).

Whether HIV-infected females with reduced GH could benefit from r-hGH replacement treatment remains unknown. At present, r-hGH treatment is not indicated in HIV infection (13, 14), except for the treatment of AIDS- and HIV-related muscle wasting in the USA (36). Thus, treating HIV-infected individuals with documented biochemical evidence of GHD should be considered off-label and inappropriate. However, recent studies suggest beneficial effects of a GHRH analog among HIV-infected patients with central fat accumulation, and this benefit on visceral fat was seen specifically in subanalyses performed among HIV-infected women enrolled in the studies (37, 38, 39).

Future studies are needed to further investigate the effects of GH and GHRH analog on body composition, BMD, and quality of life among HIV-infected women and especially among women with impairment of the GH axis. Whether biochemical GHD should be considered as a true clinical GHD in HIV-infected females and should be treated remains unsolved. Probably, reduced GH secretory response observed in this study is functional, but the differences between HIV patients with and without peak GH response in terms of the quality of life and BMD speak in favor of the presence of clinical conditions similar to those of patients who suffer from true GHD in these patients with HIV. Hence, it is possible that a subset of HIV-infected patients with significant reductions in GH secretory response may benefit from augmentation of the GH axis as an endpoint, to improve quality of life and BMD, in addition to VAT. As a word of caution, recent data suggest that adiposity in

HIV-infected patients with GHD was worsened after the withdrawal of physiological doses of r-hGH (40), perhaps due to prolonged suppression of the GH axis from exogenous GH. In distinction, this effect was not seen with GHRH analog, which does not suppress pituitary function. Our results may be of help in identifying patients who may benefit from augmentation of the GH axis.

This study not only has some strengths, such as the great number of participants and the lack of obese women enrolled, but also has several limitations that are listed below. As estrogens may positively modulate GH secretion, the first limitation is that the subjects enrolled in the study were tested for GH secretion irrespective of their phase of the menstrual cycle and the sample we studied also included postmenopausal women. The lack of FFA measurement represents the second main limitation of this study, as FFAs decrease GH secretion and impair GH response to secretagogue in both HIV-infected patients (9) and HIV-uninfected subjects (41). Thirdly, GHRH+Arg assesses the integrity of the GH pituitary reserve, thus in this setting the hypothalamic function could not be tested. Fourthly, CT scan was not performed in controls, thus limiting the chance to compare VAT among HIV-infected and -uninfected subjects. Finally, the lack of information on the morphology of the hypothalamic–pituitary area does not allow us to determine whether some of the HIV-infected women with an impaired GH peak suffered from other diseases causing GHD.

In conclusion, our data suggest reduced GH peak and AUC after GHRH+Arg testing in HIV compared with matched controls and suggests a lesser role of VAT in influencing GH response to GHRH+Arg among HIV-infected lipoatrophic, nonobese women. Furthermore, this study suggests that $\sim 20\%$ of HIV-infected females with lipoatrophy fail to respond to GHRH+Arg using previously reported cutoffs and these patients exhibit low BMD and poor quality of life typical of true GHD, although our data do not permit us to formally establish this diagnosis in our subjects. Similarly, the optimal cutoff for the diagnosis of reduced GH secretory response in HIV infection remains uncertain, even though using more restrictive cutoffs may result in less misclassification. As changes in body composition typical of HIV-infected women with lipodystrophy do not fully explain reduced GH secretory response in the patients studied and a primary hypothalamic–pituitary disease causing true GHD in such a large percentage is unlikely, our data suggest that other factors including the HIV virus, HAART treatment, and health status might affect hypothalamic–pituitary function in HIV and should be further investigated. This study provides new information on GH status in HIV-infected women. Further studies are needed to investigate the potential utility and optimal targeting of r-hGH and GHRH analog in HIV-infected women.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-11-0829>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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