Metabolic regulation of growth hormone by free fatty acids, somatostatin, and ghrelin in HIV-lipodystrophy

Polyxeni Koutkia, Gary Meininguer, Bridget Canavan, Jeff Breu, and Steven Grinspoon. Metabolic regulation of growth hormone by free fatty acids, somatostatin, and ghrelin in HIV-lipodystrophy. Am J Physiol Endocrinol Metab 286: E296–E303, 2004. First published October 14, 2003; 10.1152/ajpendo.00335.2003.—Human immunodeficiency virus (HIV)-lipodystrophy is a syndrome characterized by changes in fat distribution and insulin resistance. Prior studies suggest markedly reduced growth hormone (GH) levels in association with excess visceral adiposity among patients with HIV-lipodystrophy. We investigated mechanisms of altered GH secretion in a population of 13 male HIV-infected patients with evidence of fat redistribution, compared with 10 HIV-nonlipodystrophic patients and 11 male healthy controls similar in age and body mass index (BMI). Although similar in BMI, the lipodystrophic group was characterized by increased visceral adiposity, free fatty acids (FFA), and insulin and reduced extremity fat. We investigated ghrelin and the effects of acute lowering of FFA by acipimox on GH responses to growth hormone-releasing hormone (GHRH). We also investigated somatostatin tone, comparing GH response to combined GHRH and arginine vs. GHRH alone with a subtraction algorithm. Our data demonstrate an equivalent number of GH pulses (4.1 ± 0.6, 4.7 ± 0.8, and 4.5 ± 0.3 pulses/12 h in the HIV-lipodystrophic, HIV-nonlipodystrophic, and healthy control groups, respectively, P > 0.05) but markedly reduced GH secretion pulse area (1.14 ± 0.27 vs. 4.67 ± 1.24 ng · ml⁻¹ · min⁻¹, P < 0.05), HIV-lipodystrophic vs. HIV-nonlipodystrophic; 1.14 ± 0.27 vs. 3.18 ± 0.92 ng · ml⁻¹ · min⁻¹, P < 0.05 HIV-lipodystrophic vs. control), GH pulse area, and GH pulse width in the HIV-lipodystrophy patients compared with the control groups. Reduced ghrelin (418 ± 46 vs. 514 ± 37 pg/ml, P < 0.05, HIV-lipodystrophic vs. HIV-nonlipodystrophic; 418 ± 46 vs. 546 ± 45 pg/ml, P < 0.05, HIV-lipodystrophic vs. control), impaired GH response to GHRH by excess FFA, and increased somatostatin tone contribute to reduced GH secretion in patients with HIV-lipodystrophy. These data provide novel insight into the metabolic regulation of GH secretion in subjects with HIV-lipodystrophy.

The physiological regulation of growth hormone (GH) is complex and occurs under the dual influence of growth hormone-releasing hormone (GHRH) and somatostatin. More recently, it has been suggested that ghrelin, a nutritionally mediated gut peptide and GH secretagogue (19), may also be an important regulator of GH secretion. GH is reduced in generalized obesity (35), and recent studies suggest that visceral fat is a critical determinant of GH secretion (6). Increased somatostatin tone is thought to contribute to reduced GH secretion in obesity, but little is known regarding the pattern of GH secretion and regulation of GH by somatostatin, GHRH, and ghrelin in lipodystrophic conditions in which total fat may be unchanged but fat distribution markedly altered.

HIV-lipodystrophy is a recently described metabolic syndrome characterized by changes in fat distribution and insulin resistance (5, 13, 14). Fat distribution changes are heterogeneous and can include reduced subcutaneous fat as well as increased visceral fat. We have previously shown decreased GH secretion in patients with HIV-lipodystrophy (26), but the mechanisms of altered GH secretion in this population remain unknown. In this study, we sought to further characterize the mechanisms of reduced GH secretion among HIV-infected patients with lipodystrophy compared with age- and body mass index (BMI)-similar control groups. We investigated GH pulse dynamics and specific mechanisms related to ghrelin, somatostatin, and GHRH. Our data suggest that increased somatostatin tone, impaired GHRH stimulation of GH by excess free fatty acids (FFA), and reduced ghrelin may contribute to the altered pattern of GH secretion in HIV-lipodystrophy.

MATERIALS AND METHODS

Inclusion and exclusion criteria. Thirty-four male subjects [23 HIV-infected and 11 healthy controls similar in age and body mass index (BMI)] were enrolled in the study between October 2001 and January 2003. HIV-infected subjects were divided into two categories, lipodystrophic and nonlipodystrophic, on the basis of evidence of fat redistribution. All HIV-infected subjects were required to be on a stable antiretroviral regimen for ≥3 mo before entrance into the study, including at least one nucleoside reverse transcriptase inhibitor and either one protease inhibitor or one nonnucleoside reverse transcriptase inhibitor. Subjects were categorized as lipodystrophic on the basis of a waist-to-hip ratio (WHR) >0.95 and evidence of fat redistribution, including increased fat under the chin, at the back of the neck, or in the abdominal, chest, or breast areas or decreased fat in the arms, legs, or face. A score for fat change (between 0 and 2) was graded for each site, and subjects with an increased WHR and lipodystrophy score ≥2.0 were characterized as lipodystrophic, whereas HIV-infected subjects with a WHR <0.95 and a lipodystrophy score of <2.0 were characterized as nonlipodystrophic. Control subjects were healthy, with a WHR <0.95. Subjects with diabetes mellitus, BMI <20 kg/m², hemoglobin <9 g/dl, and use of GH, GHRH, oral or parenteral glucocorticoids, megesterol acetate, or antidiabetic agents within the 3 mo before study initiation were excluded.

Written, informed consent was obtained from each subject before testing, in accordance with the Committee on the Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology and the Subcommittee on Human Studies at the Massachusetts Gen...
eral Hospital. Each subject returned for a series of two outpatient and two inpatient visits to the General Clinical Research Center (GCRC) at Massachusetts General Hospital (Fig. 1). Partial data were obtained in three patients in whom not all testing was completed.

Screening outpatient visit. After a 12-h overnight fast, subjects reported to the GCRC for a screening visit, at which time eligibility was determined on the basis of a fasting blood glucose level <126 mg/dl and a 2-h glucose level <200 mg/dl on 75-g oral glucose tolerance test.

GH assessment. Subjects returned at 0700 to the GCRC on each of 2 days separated by 1 wk for testing with either GHRH alone or a combined GHRH-arginine stimulation test (Fig. 1). The order of testing was randomized between visits; e.g., subjects were randomized to receive GHRH alone on the first visit and then combined GHRH and arginine on the second visit or vice versa. GHRH stimulation testing [GHRH-1-29 (Geref; Serono, Norwell, MA), 1 μg/kg iv bolus] was performed according to protocol, with GH levels collected at −15, 0, 15, 30, 45, 60, 90, and 120 min after the bolus of GHRH was given. GHRH-arginine stimulation testing was performed according to standardized protocol [GHRH-1-29 (Geref; Serono), 1 μg/kg iv bolus along with simultaneous administration of 0.5 g/kg arginine hydrochloride (maximum dose 30 g) iv over 30 min]. GH levels were collected at −15, 0, 15, 30, 45, 60, 90, and 120 min after GHRH administration.

Subjects returned for the fourth and fifth visits (inpatient) separated from each other by 1 wk. For each visit, subjects reported to the GCRC at 1700 for an overnight fast, with sampling performed every 20 min beginning at 1900 and ending at 0740 the next morning. Subjects were not permitted to eat after 1800 on the evening before and during frequent sampling. Subjects were randomized to receive either placebo or acipimox tablets at each visit [two 250-mg tablets of acipimox or placebo (total 500 mg) at 0200 and again at 0600]. Subjects who initially were randomized to receive acipimox for the fourth visit received placebo tablets for the fifth visit and vice versa per randomization. At 0800 (time = 0 min) on each visit, after overnight frequent sampling, a standard GH stimulation test [GHRH-1-29 (Geref; Serono), 1 μg/kg iv bolus] was performed, and GH levels were collected at 30, 60, 90, and 120 min after GHRH administration (Fig. 1). Acipimox was well tolerated by all subjects. One subject developed moderate flushing ~45 min after receiving acipimox, but this resolved spontaneously 2 h later.

Body composition analysis. Height, weight, and BMI were determined in the fasting state during the first outpatient visit. Cross-sectional abdominal computed tomography scanning was performed to assess the distribution of subcutaneous (SAT) and visceral abdominal fat (VAT). A lateral scout image was obtained to identify the level of the L4 pedicle, which served as a landmark for the single-slice image. Scan parameters for each image were standardized (144 cm table height, 80 kV, 70 mA, 2 s, 1-cm slice thickness). Fat attenuation was then determined (4). Extremity fat was determined by in vitro enzymatic colorimetric assay kit (Wako Chemicals, Richmond, VA). The intra-assay CV for fatty acids ranged from 1.1 to 2.7%. The published normal range for fatty acids is 0.1–0.6 mmol/l.

Biochemical and immunological function. Fasting glucose, insulin, ghrelin, FFA, triglyceride, CD4 cell count, and viral load were determined on the morning of the first outpatient visit before any stimulation testing.

Laboratory methods. GH was measured by two-site radioimmunometric assay with an intra-assay coefficient of variation (CV) of 4.4% (Corning; Nichols Institute Diagnostics, San Juan Capistrano, CA). The interassay CV was 6.6%. The sensitivity of the assay was determined to be 0.05 ng/ml. IGF-I was measured by two-site radioimmunometric assay with an intra-assay CV of 4.9% (Diagnostics Systems Laboratory, Webster, TX). The interassay CV was 5.1%. The sensitivity of the assay was determined to be 2.6 ng/ml.

Ghrelin was measured by RIA (Phoenix Pharmaceuticals, Belmont, CA). The RIA uses 125I-labeled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against full-length, octonoylated human ghrelin as a detection probe that recognizes both the octanoyl and desoctanoyl forms of the hormone. Ghrelin levels reported in this study were assayed from serum samples. The lower limit of detection for this assay in our laboratory was 80 pg/ml. In our laboratory, the intra-assay CV was 7.8% and the interassay CV was <10%. This assay measures total ghrelin and does not specifically assess bioactive ghrelin concentration.

Nonesterified fatty acid concentrations were measured by using an in vitro enzymatic colorimetric assay kit (Wako Chemicals, Richmond, VA). The intra-assay CV for fatty acids ranged from 1.1 to 2.7%. The published normal range for fatty acids is 0.1–0.6 mmol/l. Insulin concentrations were measured in serum by RIA (Diagnostic Products, Los Angeles, CA). The intra- and interassay CVs range from 4.7 to 7.7 and 5.5 to 9.2%, respectively. Glucose and triglyceride concentrations were measured by standard techniques.

The CD4 count was determined by flow cytometry (Becton Dickinson Immunocytometry Systems, San Jose, CA), and the HIV viral load was determined by ultrasensitive assay (AmpliCord HIV-1 Monitor Assay; Roche Molecular Systems, Indianapolis, IN), with limits of detection of 50–75,000 copies/ml.

Pulse and deconvolution analysis: pulse and cluster programs. To assess GH pulsatility, we used Cluster, a largely model-free computerized pulse analysis algorithm to identify statistically significant pulses in relation to dose-dependent measurement error in each hormone time series (33). In performing the analysis, we specified individual test cluster sizes for the nadir and peak width of 2 (2 × 2), a minimum and maximum intraseries CV, a t-statistic to identify significant increase, and a t-statistic to define a significant decrease (32). A CV of 4.4%, the intra-assay CV for our GH assay, was used in the settings of the program. Information about the secretion of the hormone into the serum and the elimination of the hormone from the serum was obtained from PULSE 2 and PULSE 4 deconvolution and pulse detection algorithms.

Statistical analysis. Demographic and pulse characteristics among the three groups were compared by ANOVA. When the P value for the overall comparison among the three groups was >0.05, t-tests were performed comparing data among individual groups (HIV-lipodystrophy vs. HIV-nonlipodystrophic vs. normal controls). Peak GH and GH area under the curve (AUC) responses to GHRH and to combined GHRH and arginine were compared among the three groups by the nonparametric Wilcoxon test. The percent increase in peak GH response [(peak GH_{GHRH} + arginine) − (peak GH_{GHRH})]/
(peak GH of GHRH) and percent increase in AUC GH responses between the two GH stimulation tests were compared by the Wilcoxon test. GH responses to GHRH were compared with the change in FFA before and after acipimox dosing. Fasting morning ghrelin was compared between the groups by the Wilcoxon test and with body composition, metabolic indexes, and GH pulsatility in univariate regression analyses.

**RESULTS**

**Subject characteristics.** Male HIV-infected patients with lipodystrophy and HIV-infected patients without lipodystrophy were compared with simultaneously recruited male controls with mean age of 38.4 ± 1.5 yr and BMI 27.1 ± 1.0 kg/m² (Table 1). Visceral fat and fasting insulin, FFA, and triglycerides were significantly increased in the HIV-lipodystrophy patients compared with the control groups (Table 1). Lower extremity fat was reduced in the HIV-lipodystrophy group (Table 1).

**GH pulse characteristics and IGF-I.** Pulse dynamics were observed in the number of GH peaks between the groups (4.1 ± 0.6, 4.7 ± 0.8, and 4.5 ± 0.3 pulses/12 h in HIV-lipodystrophic, HIV-nonlipodystrophic, and healthy control groups, respectively; Table 2). The mean GH secretion pulse area was significantly different among the three groups (P < 0.03) and was significantly lower among the HIV-infected patients with lipodystrophy compared with the HIV-nonlipodystrophic and healthy control groups (1.4 ± 0.27 mg·ml⁻¹·min, P < 0.05, HIV-lipodystrophic vs. HIV-nonlipodystrophic; 1.14 ± 0.27 vs. 3.18 ± 0.92 mg·ml⁻¹·min, P < 0.05, HIV-lipodystrophic vs. control; Table 2). The mean peak width and height were also significantly reduced in the HIV-lipodystrophic patients compared with the other groups (Table 2). IGF-I levels were 31% lower in the lipodystrophic group compared with the other groups (P = 0.08) and 31% lower in the lipodystrophic group compared with the nonlipodystrophic group (P = 0.05).

**Subtraction algorithm to determine somatostatin tone.** The peak GH response to GHRH was significantly lower in patients with HIV-lipodystrophy compared with HIV-nonlipodystrophic patients (2.5 ± 0.5 vs. 12.8 ± 4.6 mg/ml, P < 0.05) and compared with control patients (2.5 ± 0.5 vs. 11.3 ± 3.8 mg/ml, P < 0.05; Table 3). The AUC GH response to GHRH

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**Table 1. Demographics of the study population**

<table>
<thead>
<tr>
<th></th>
<th>HIV-Infected With Lipodystrophy (n=13)</th>
<th>HIV-Infected Without Lipodystrophy (n=10)</th>
<th>Controls (n=11)</th>
<th>P Value for Overall Comparison by ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>42.6±1.6</td>
<td>39.2±1.7</td>
<td>38.4±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.1±0.7</td>
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<td>27.1±1.0</td>
<td>NS</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.99±0.01†</td>
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</tr>
<tr>
<td>Subcutaneous fat, cm²</td>
<td>237±32</td>
<td>170±17</td>
<td>254±31</td>
<td>NS</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>197±19†</td>
<td>66±10</td>
<td>94±13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lower extremity fat by DEXA, kg</td>
<td>43.3±6.7†</td>
<td>57.7±4.7</td>
<td>70.4±9.2</td>
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</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>22.1±3.9†</td>
<td>6.4±1.7</td>
<td>8.2±1.0</td>
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</tr>
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<td>Insulin area under the curve, μU·ml⁻¹·min</td>
<td>12.693±2.486†</td>
<td>5.442±780</td>
<td>5.105±513</td>
<td>0.005</td>
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<tr>
<td>Fasting glucose, mg/dl</td>
<td>99±3</td>
<td>95±3</td>
<td>96±3</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose area under the curve, mg·dl⁻¹·min</td>
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<td>15.713±1.047</td>
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<td>NS</td>
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<td>FFA, mcg/l</td>
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<td>0.38±0.03</td>
<td>0.40±0.04</td>
<td>0.02</td>
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<td>Triglycerides, mg/dl</td>
<td>333±90*</td>
<td>131±19</td>
<td>123±27</td>
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<tr>
<td>IGF-I, ng/ml</td>
<td>238±28</td>
<td>343±46</td>
<td>320±36</td>
<td>NS</td>
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<tr>
<td>CD4 count, cells/mm³</td>
<td>551±45</td>
<td>403±77</td>
<td>NS</td>
<td></td>
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<td>Viral load, log₁₀ (copies/ml)</td>
<td>2.5±0.3</td>
<td>2.2±0.3</td>
<td>NS</td>
<td></td>
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<tr>
<td>% On PI-containing regimen</td>
<td>85</td>
<td>80</td>
<td>NS</td>
<td></td>
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<tr>
<td>% On NRTI-containing regimen</td>
<td>100</td>
<td>100</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>% On NNRTI-containing regimen</td>
<td>38</td>
<td>20</td>
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</tbody>
</table>

Values are means ± SE. HIV, human immunodeficiency virus; DEXA, dual-energy X-ray absorptiometry; FFA, free fatty acids; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; NNRTI, non-NRTI. *P < 0.05 vs. Controls; †P < 0.05 vs. no lipodystrophy.
was also significantly reduced in the lipodystrophy group compared with the two control groups (Table 3). Percent increase in peak GH response [(peak \( \Delta \text{GH} \)) + arginine]  - [(peak \( \Delta \text{GH} \))] / (peak \( \Delta \text{GH} \)) and percent increase in AUC GH responses between the two GH stimulation tests were significantly greater in the HIV-lipodystrophy group (Table 3).

Responses to acipimox. Fasting morning FFA were significantly increased in the HIV-lipodystrophy group compared with HIV-nonlipodystrophic and control subjects before acipimox. The fasting FFA level decreased significantly and equivalently among all three groups in response to acipimox (Fig. 2). The change in peak GH response to GHRH before and after acipimox was significantly correlated with the change in FFA in the HIV-lipodystrophic group (\( r = -0.61, P = 0.04 \)), such that response to GHRH increased most in association with the largest decrease in FFA. In contrast, the changes in FFA in response to acipimox were not associated with the changes in GH responsiveness to GHRH before and after acipimox in either the HIV-nonlipodystrophy group or in the healthy control group (Table 4).

Ghrelin and correlation with GH pulse dynamics. The fasting ghrelin level was significantly lower among the HIV-lipodystrophy group compared with the HIV-nonlipodystrophic and control groups (Fig. 3). Fasting morning ghrelin level also correlated highly with GH mean secretion (\( r = 0.73, P = 0.01 \)) and GH pulse area (\( r = 0.59 \) and \( P = 0.05 \)) in the HIV-lipodystrophic but not in the other groups (Table 5).

Fasting morning ghrelin correlated significantly with number of GH peaks among the HIV-infected subjects (\( r = 0.54, P = 0.01 \)) and even more strongly among the HIV-lipodystrophy group (\( r = 0.76, P < 0.01 \)). In contrast, no association was seen between ghrelin and GH pulsatility in the normal control group (Table 5). There was a strong negative correlation between ghrelin and fasting insulin (\( r = -0.57, P < 0.01 \)) and between ghrelin and insulin AUC (\( r = -0.51, P = 0.02 \)) in the HIV-infected individuals.

**DISCUSSION**

In this study, we investigated the mechanisms of decreased GH secretion in HIV-lipodystrophic patients. Significant fat distribution and insulin resistance occur in HIV-infected patients with lipodystrophy (5, 13, 14), but such changes can be heterogeneous. In a previous study, we demonstrated reduced GH secretion in patients with HIV-lipodystrophy, demonstrating that GH was decreased in association with excess visceral fat in this population (26). Similarly, we recruited HIV-infected patients with lipodystrophy for the present study, and subjects were similar in age and BMI to HIV-nonlipodystrophic patients and healthy controls but demonstrated markedly increased visceral fat and insulin compared with the other control groups. In contrast, extremity fat was reduced, suggesting a model of fat redistribution rather than generalized obesity.

Reduced GH secretion has been demonstrated in association with excess visceral adiposity in non-HIV-infected patients (6), and we (26) recently demonstrated that increased visceral adiposity predicted low GH concentrations in multivariate regression modeling controlling for indexes of obesity, subcutaneous fat, and overall adiposity in patients with HIV-lipodystrophy. The use of both an HIV-nonlipodystrophic group matched in terms of antiretroviral therapies and a control group helped us to control for the effects of HIV and antiretroviral therapy per se and focus on mechanisms of...
GH regulation related to fat redistribution and metabolic dysregulation. We did not study HIV-infected patients with severe primary lipatrophy, and different results might be seen in that patient population. In contrast, we investigated patients with extreme visceral adiposity but relatively normal weight compared with previous studies of GH regulation in severe generalized obesity (11). To our knowledge, prior studies have not compared with previous studies of GH regulation in severe generalized obesity. In this study, we simultaneously assessed the pulsatile secretion of GH. These factors may influence the synthesis and secretion of GH either directly via stimulation or inhibition of pituitary somatotrophs or indirectly via GHRH and somatostatin release from the hypothalamus or by endogenous growth hormone-releasing peptides that affect the hypothalamus and/or pituitary (29). Ghrelin, a gut peptide, was recently discovered to be a natural GH secretagogue regulated by food intake (19).

Pulsatile release of GHRH is responsible for the pulsatile pattern of GH secretion observed in humans (31, 34). The frequency and amplitude of pulsatile GHRH release affect not only the pulsatile pattern of GH release but also the overall GH concentration (17). In contrast, somatostatin, a peptide produced primarily in the periventricular and medial preoptic areas of the hypothalamus, tonically inhibits GH release from the pituitary somatotrophs (1, 9).

In obese men, the mean 24-h serum GH concentration and GH pulse frequency are reduced, and daytime GH interpulse interval is lengthened compared with nonobese age-matched controls. In contrast, GH pulse amplitude is not significantly different between obese and nonobese groups (31). Furthermore, somatostatin tone was increased on the basis of the results of a subtraction algorithm, comparing the percent change in peak GH response to combined GHRH plus arginine and GHRH alone. FFA were increased in the lipodystrophic group, and GH response to GHRH was increased in proportion to the decrease in FFA by acipimox, suggesting that increased FFA inhibits GH response to GHRH in the lipodystrophic patients (Fig. 4). We also demonstrated that ghrelin is reduced in the HIV-lipodystrophic group compared with control subjects.

Changes in fat redistribution might occur due to HIV disease or exposure to antiretroviral medications. Data from our study suggest that metabolic dysregulation in this setting, e.g., insulin resistance and increased FFA, may contribute to decreased GH through a number of different mechanisms, e.g., somatostatin tone, reduced GH response to GHRH, and decreased ghrelin. In turn, decreased GH resulting from any of the mechanisms elucidated in this study may contribute to visceral adiposity and insulin resistance (18), creating a vicious cycle of metabolic dysregulation.

In humans, the neuroendocrine control of pulsatile GH secretion is poorly understood. A number of factors can modulate the pulsatile secretion of GH. These factors may influence the synthesis and secretion of GH either directly via stimulation or inhibition of pituitary somatotrophs or indirectly via GHRH and somatostatin release from the hypothalamus or by endogenous growth hormone-releasing peptides that affect the hypothalamus and/or pituitary (29). Ghrelin, a gut peptide, was recently discovered to be a natural GH secretagogue regulated by food intake (19).

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Table 5. Ghrelin correlation with GH pulse characteristics, body composition, and metabolic indexes

<table>
<thead>
<tr>
<th></th>
<th>All HIV-Infected Individuals (n=23)</th>
<th>HIV-Infected With Lipodystrophy (n=13)</th>
<th>HIV-Infected Without Lipodystrophy (n=9)</th>
<th>Controls (n=11)</th>
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<tbody>
<tr>
<td>BMI, kg/m²</td>
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<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
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<td></td>
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<td>0.28</td>
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<td>Subcutaneous fat, cm²</td>
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<td>Insulin AUC, μU·ml⁻¹·min⁻¹</td>
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<td>GH pulse characteristics</td>
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<tr>
<td>Peak/12 h</td>
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<td>Mean secretion pulse area, ng·ml⁻¹·min⁻¹</td>
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<td>Peak height, ng/ml</td>
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<td>Area, ng·ml⁻¹·min⁻¹</td>
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</tr>
<tr>
<td>Nadir, ng/ml</td>
<td>0.05</td>
<td>0.84</td>
<td>0.10</td>
<td>0.79</td>
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more, obese subjects have a significantly blunted GH response
to the GH secretagogues GHRH and arginine, alone or in
combination, compared with nonobese controls (11). Veldhuis
et al. (35) demonstrated that the degree of body fatness was
associated inversely with indexes of GH secretion. In contrast
to a model of generalized obesity, we investigated patients with
fat redistribution and excess visceral fat but reduced extremity
fat.

Somatostatin inhibits GH release from the pituitary and is
thought to be a potential mechanism for predicting reduced GH
secretion in generalized obesity (11). Increased somatostatin
tone might contribute to reduced GH concentration area, re-
duced GH pulse amplitude, and, ultimately, reduced mean GH
concentrations with preserved GH pulse frequency in patients
with HIV-lipodystrophy (26). To ascertain endogenous soma-
tostatin tone, indirect techniques are needed, since currently no
selective somatostatin receptor inhibitor exists for use in hu-
mans. Arginine is an amino acid that is believed to increase GH
by suppression of endogenous somatostatin secretion (1, 9, 10).
Investigation of somatostatin and GHRH have not, to our
knowledge, been performed in subjects with excess visceral
adiposity and fat redistribution.

Several groups (1, 21) have shown that arginine potentiates
maximal GHRH-stimulated GH release, suggesting that argi-
nine exerts its effects on GH secretion by decreasing soma-
tostatin tone rather than increasing GHRH secretion. One
experimental technique to determine the role of somatostatin
tone in lowering GH levels in HIV-lipodystrophy is to compare
the difference in GH release in response to stimulation by
GHRH vs. combined GHRH-arginine (3, 9, 11). Arginine is
thought to increase GH by inhibition of somatostatin tone and
is therefore expected to significantly augment the stimulating
response to GHRH alone when somatostatin tone is increased
(9, 11). For example, arginine has been shown to augment the
response to GHRH in generalized obesity through a postulated
inhibition of endogenous somatostatin tone (11).

In this study, we investigated the role of somatostatin as a
potential factor contributing to the observed differences in GH
pulse dynamics among the lipodystrophic patients by use of a
subtraction algorithm to evaluate indirectly somatostatin tone.
The percent increase in stimulated GH resulting from the
combined GHRH-arginine test compared with GHRH alone
was largest in the lipodystrophy group, consistent with our
hypothesis of increased somatostatin tone in the lipodystrophic
group. Our data extend the prior data of Ghigo et al. (11),
demonstrating increased somatostatin tone in the setting of
severe generalized obesity, and suggest that increased soma-
tostatin tone may also contribute to reduced GH excretion in
patients with fat redistribution and visceral obesity.

A second possible mechanism to explain decreased GH
secretion is altered GHRH pulsatility or GH responsivity to
GHRH at the pituitary. In this regard, we hypothesized de-
creased GH responsivity to GHRH, as GH pulsatility was not
reduced. We further hypothesized that increased FFA resulting
from visceral obesity would blunt GH responses to GHRH as
a potential mechanism of reduced GH secretion. Previous
studies in HIV-lipodystrophic patients with visceral adiposity
demonstrate increased FFA and lipolysis rates (12, 13, 15).
Moreover, Hadigan et al. (15) demonstrated that increased FFA
may mediate reduced insulin sensitivity in this group of pa-
tients by using acipimox to decrease FFA and acutely increase
insulin sensitivity. In this study, we used acipimox as a phys-
iological probe to acutely decrease FFA and investigate the
effects of FFA manipulation on GH responsivity to GHRH.
Pontiroli et al. (25) have shown in a randomized placebo-
controlled trial of acipimox (500 mg) given to normal healthy
subjects that GH response to GHRH was significantly greater
in those who were pretreated with acipimox. Several studies
have suggested that the mechanism by which the increased
amounts of FFA cause diminished GH secretion is by increas-
ing endogenous somatostatin tone (16, 20, 21). However, other
studies performed in animals suggest that FFA impair GH
release directly at the pituitary gland (2).

Our data demonstrate that the change in FFA in response to
acipimox was highly significantly correlated with the change in
peak GH responsivity to GHRH in the HIV-lipodystrophic
subjects but not in the other groups. Our data suggest that
excess FFA in the setting of fat redistribution and insulin

Fig. 4. Potential schema for the mechanisms
of reduced GH secretion in HIV-lipodystro-
phy.
resistance may impair GH responsiveness to GHRH. The tight regulation between reduction in FFA and increase in GH responsiveness to GHRH in the lipodystrophic group supports this hypothesis, but further studies of the quantitative effects of higher ambient FFA concentrations in HIV-lipodystrophy are needed, for example, to determine whether there is a threshold for FFA effects on GH. Impaired GH response to GHRH would also explain the findings of normal GH pulsatility with decreased GH pulse width, pulse area, and overall secretion rate. It is also possible that increased FFA contribute to increased somatostatin tone in our subjects, as these mechanisms are not mutually exclusive. Furthermore, as suggested by Sekhar et al. (26a), increased lipolysis may occur in HIV-infected patients with more severe lipoatrophy. HIV-lipodystrophy may not be a single syndrome, and the relationship of visceral adiposity and subcutaneous fat loss is unclear. It is possible that excess FFA may alter GH secretion in the setting of severe lipodystrophy without visceral adiposity, and further studies of GH secretion in this group would be interesting. Furthermore, FFA may not be increased among all patients with HIV-lipodystrophy, and further studies of GH secretion in such patients would also be interesting. In addition, studies of HIV-infected women are necessary to investigate potential sex differences in GH regulation.

A third possible mechanism of reduced GH secretion in lipodystrophic patients is reduced ghrelin. Ghrelin is a recently discovered gut peptide that is the endogenous ligand for the GH secretagogue receptor (28). Ghrelin is nutritionally regulated and decreases with obesity (27), increases in starvation and before a meal, falls rapidly after food intake, and strongly stimulates GH secretion in humans (7). It has been demonstrated that circulating GH levels are reduced in obese subjects (30) who are insulin resistant and hyperinsulimemic (8). Recent data suggest that ghrelin is an important regulator of GH secretion during fasting as well as in response to caloric intake (24). In this study, we demonstrate reduced ghrelin in the HIV-lipodystrophy group in association with reduced mean GH pulse area, GH secretion, and increased insulin. In fact, our data suggest that insulin concentration, more than BMI, is associated with reduced ghrelin. It is possible that insulin-induced suppression of ghrelin may contribute to reduced GH secretion in HIV-lipodystrophy. However, the relationship between insulin and ghrelin is unclear in normal physiology, and it remains uncertain whether insulin per se or pattern of macronutrient ingestion affects ghrelin. Alternatively, increased somatostatin tone may contribute to low ghrelin concentration. Low ghrelin may be a marker of metabolic dysregulation and altered body fat without specifically contributing to low GH. Determination of a single fasting ghrelin level may be inadequate, and further studies are necessary to determine meal-related ghrelin responses in relationship to GH in HIV-lipodystrophy. In addition, our study was limited to the determination of total ghrelin, whereas measures of bioactive ghrelin may also be useful. Further studies will be needed to investigate the specific role of reduced ghrelin in mediating low GH in conditions of visceral adiposity.

Our data demonstrating reduced GH secretion, peak area, and preserved GH pulsatility are similar to those of Magiakou et al. (22). Although urine free cortisol levels and other tests of the hypothalamo-pituitary-adrenal axis have not been shown to be abnormal in HIV-lipodystrophy (23, 26), it is possible that patients with HIV-lipodystrophy may have glucocorticoid hypersensitivity at the level of the glucocorticoid receptor. Further studies are needed to investigate whether there is a relationship between altered glucocorticoid and GH dynamics in this population.

In conclusion, this study helps elucidate the physiological mechanisms of reduced GH secretion in HIV-lipodystrophy. In a group of lipodystrophic patients with visceral obesity and fat redistribution, we demonstrate an equivalent number of GH pulses but markedly reduced GH secretion, pulse area, and GH pulse width compared with control subjects. Our data suggest increased somatostatin and an effect of excess FFA to impair GH response to GHRH. Furthermore, we demonstrate low ghrelin, which may also contribute to low GH in this population. Taken together, these data suggest a complex schema whereby alterations in insulin and fatty acids may affect GH secretion in HIV-lipodystrophy.

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GRANTS

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