Effects of Growth Hormone Releasing Hormone Treatment on Fat Redistribution, Cardiovascular Indices and Growth Hormone Secretion in HIV Lipodystrophy

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Detailed Protocol
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I. Background and Significance

HIV-infected patients demonstrate significant metabolic and body composition abnormalities. These changes include insulin resistance, dyslipidemia, and body composition changes. Importantly, recent data suggest that cardiovascular disease is increased among HIV-infected patients, and can be linked to specific abnormalities, such as dyslipidemia. The body composition changes include lipoatrophy in a majority of patients, but also increased visceral fat accumulation in a large number of such patients. Although there remains significant controversy regarding the etiology of these changes and whether changes in subcutaneous and visceral fat are linked, prospective longitudinal data in ARV naïve patients starting antiretroviral therapy demonstrate increased truncal adiposity and fat loss, and these changes are often concordant.

Significant data from non HIV-infected patients demonstrate that increased abdominal fat is linked to increased rates of acute myocardial infarction (AMI). Our group and many others have shown that increased truncal adiposity among the HIV-infected patients is primarily composed of excess visceral fat, with a relative reduction in subcutaneous fat.

In patients with HIV and visceral fat accumulation, mean overnight GH secretion is significantly reduced by almost 50% compared to HIV-infected patients without fat redistribution and also non HIV-infected patients of similar BMI. In multivariate regression analysis, including BMI, total fat and visceral fat as independent variables, visceral adiposity was the most significantly associated with reduced GH secretion. Thus, a vicious cycle is created, whereby the increase in visceral fat seen among HIV-infected patients, results in reduced GH secretion, which can result in further increases in visceral adiposity. In subsequent studies, investigating detailed response to GHRH+arginine, we demonstrated reduced peak GH secretion to GHRH+arginine in association with increased VAT, among HIV-infected patients with changes in fat distribution. In addition, HIV-infected patients with fat accumulation were clearly capable of responding to GHRH alone, but this response was reduced. Careful studies of GH pulsatility demonstrate that HIV-infected patients have a similar number of discrete overnight GH pulses, but reduced GH pulse amplitude.

A number of lines of evidence suggest that relative GH deficiency, as is seen among HIV-infected patients with central fat accumulation, may result in increased cardiovascular risk. Patients with true GH deficiency exhibit a phenotype similar to metabolic syndrome, including increased visceral fat, elevated lipids, and elevated blood pressure. GH deficient patients have an increased rate of premature mortality from cardiovascular disease, which may be related to an abnormal lipid profile or other factors, including endothelial dysfunction and increased inflammatory indices. Importantly, adults with partial GH deficiency exhibit increased waist to
hip ratio, reduced lean mass and increased truncal fat mass\textsuperscript{14}. Data from our group suggest increased waist circumference, triglyceride, and blood pressure in HIV-infected adults with reduced GH secretion\textsuperscript{15}. Furthermore, in GH deficient adults, treatment with GH reduces CV risk, by improving lipid parameters, reducing visceral fat and decreasing inflammatory markers\textsuperscript{16}. GH treatment has also been shown to reduce carotid IMT in patients with GH deficiency\textsuperscript{17}.

The mechanisms of vascular disease in subjects with reduced GH secretion are not known but may relate to traditional risk factors including insulin resistance, dyslipidemia, or to other factors, including inflammation and impaired fibrinolysis\textsuperscript{12, 18-22}. It is hypothesized that impaired glucose tolerance, hyperinsulinemia, and hypertriglyceridemia occur in part due to abnormal body fat distribution among patients with reduced GH secretion. Fasting insulin levels above the normal reference range are seen in association with increased abdominal adiposity in adults with GHD\textsuperscript{23}. Johansson et al. demonstrated decreased glucose infusion requirement in adults with GHD compared with that in matched controls, indicating reduced insulin sensitivity\textsuperscript{24}. Similarly, our group has demonstrated that reduced GH secretion in HIV-infected patients is associated with increased insulin and glucose\textsuperscript{15}. Preliminary evidence in non HIV-infected patients suggests effects of GH not only to improve visceral adiposity, but also to improve ectopic fat distribution, for example in the liver and muscle, which may contribute to improved insulin resistance over the long-term. Administration of ultra low doses of GH to viscerally obese women was shown to improve hepatic fat, as demonstrated by reduced attenuation on CT scan\textsuperscript{25}.

Dyslipidemia is as also common among patients with reduced GH secretion. Salomon et al. demonstrated elevated levels of total and LDL cholesterol in patients with GHD compared to control subjects\textsuperscript{23, 26}. Abdu et al. compared the lipid profile and coronary risk predicted by the Framingham Heart Study equation in GH-deficient hypopituitary patients and healthy age and gender-matched controls. In this study Abdu et al. evaluated 50 adult-onset GH deficient hypopituitary patients on appropriate conventional hormone replacement and 45 controls, matched for age, gender and smoking habit. Coronary risk was calculated for each individual from age, gender, systolic blood pressure, total and HDL cholesterol, smoking habit and presence of diabetes using the Framingham equation. Relative risk of coronary heart disease was significantly higher in GHD hypopituitary adults who presented with higher LDL lipid levels than the control population\textsuperscript{13}. In the current study we hypothesize that long-term GHRH\textsuperscript{1-44} will significantly improve the lipid profile, including detailed markers of lipoproteins and LDL particle size, among relatively GH deficient HIV-infected patients with central fat accumulation.

In addition to traditional risk factors, reduced GH secretion is also associated with increased inflammatory cardiovascular markers, endothelial dysfunction and increased IMT. Miller et al. evaluated 15 healthy female volunteers who were divided into two groups of comparable BMI with low and high truncal fat. There was a strong inverse association between mean 24-h GH and both truncal fat and cardiovascular risk markers, including high-sensitivity CRP, suggesting that decreased GH secretion may be associated with increased cardiovascular risk markers in this population\textsuperscript{27}. Indeed, data from our group demonstrate that increased blood pressure and CRP are associated with reduced GH secretion in HIV-infected patients\textsuperscript{15}. Increased PAI-1 activity is also associated with adult GH deficiency\textsuperscript{28, 29}, and decreases with GH therapy. Furthermore, GH deficiency is associated with increased intimal-media thickness (IMT) at major arteries\textsuperscript{30-33}. 
Blood vessel intimal media-thickening is considered as one of the earliest morphological changes in the arterial wall in the process of atherogenesis 34. Carotid IMT is considered an independent predictor of acute myocardial infarction 35, 36 and cerebrovascular disease 37. In the current proposal, we will examine detailed inflammatory measures of increased CVD risk in HIV-infected patients in relationship to GH secretion, using detailed pulse analysis, to determine the metabolic and CVD risk factors independently associated with reduced GH secretion in the HIV population.

Recent data suggest that GH administration improves atherosclerotic indices in adults with deficient GH secretion 38, 39. Abdu et al. examined the effect of 12 months of GH therapy on endothelial function, CRP and coronary risk. They reported that biophysical testing of endothelial function improved after 12 months of GH therapy. Calculated coronary risk decreased mainly due to reduction in systolic and diastolic blood pressure and increased HDL-cholesterol 40. GH replacement in adult GH deficient patients improves endothelial function in studies ranging from 3-12 months in duration 40-42. Pfeifer et al. demonstrated that improvements in endothelial function seen at 3 months were sustained over 18 months of GH treatment 43. Soares et al. demonstrated that carotid IMT was increased compared to age, gender and BMI matched adult control patients and that treatment with GH for 24 months improved IMT 17. In some shorter-term studies, treatment with GH over 6 months was not shown to significantly improve carotid IMT in adults with GHD 44, but treatment with GH over one year was shown to significantly improve IMT 45. In contrast, other studies demonstrate improvement in carotid IMT by 6 months with even earlier improvement in flow mediated dilation at 3 months after initiation of GH 43. Cardiac function and performance is improved after GH replacement therapy 46 in adult GH deficient patients 47-50, commensurate with improvements in traditional risk factors, such as dyslipidemia 51. These data obtained in GH deficient populations suggest that increasing endogenous GH secretion in relatively GH deficient, HIV-infected patients will improve CVD risk indices.

Taken together, the above data pose a paradox. Significant evidence exists to suggest that patients with reduced GH secretion, including those HIV-infected patients with relative GHD due to central fat accumulation, exhibit insulin resistance and dyslipidemia, yet exogenous GH administration may potentially aggravate some of these very conditions, likely as an effect of its nonphysiologic, nonpulsatile mode of administration. In contrast, we speculate, due to its physiological effects, GHRH will actually improve insulin resistance, along with other parameters of relative GHD, including dyslipidemia, IMCL and hepatic fat accumulation, and abnormal inflammatory cytokines and potentially increased IMT. This hypothesis is based on the knowledge that GHRH can achieve physiological changes in GH pulsatility, and will simply help to restore endogenous GH secretion, without pharmacological effects. This will help to reverse the relative GH deficient state, reversing the attendant insulin resistance, and related accumulations of visceral adiposity, IMCL and hepatic fat. In the current study, we will investigate whether the increased risk of CVD in HIV-infected patients with central fat accumulation is in part due to GH deficiency and whether physiologic augmentation of GH secretion with GHRH 1-44 improves CVD parameters in this population, such as IMT and inflammatory markers, and may even improve insulin sensitivity in the long-term.
Several strategies that target a reduction in abdominal fat in obese subjects demonstrate normalization of GH or IGF-I levels following treatment. Dietary weight loss restores 24-hour GH profiles and IGF-I levels in young obese subjects\textsuperscript{52} and severe dietary weight loss improved GH responses to hyperinsulinemic glucose clamp\textsuperscript{53}. Surgery also yields favorable results, as bariatric surgery increases GH and IGF-I levels to the normal range\textsuperscript{54}. These data suggest that the pituitary and hypothalamus of obese subjects remain intact despite functional GH deficiency, and thus the relative GH deficiency is both secondary and reversible in patients with VAT accumulation. This important concept suggests that improvement of visceral fat will increase endogenous GH secretion, which may in turn further reduce visceral adiposity, i.e., that the vicious cycle can be broken by an intervention that reduces visceral fat. Importantly, though, treatment with GH per se, may limit this effect, as it is suppressive to endogenous GH, whereas GHRH is stimulatory. Exogenous GHRH can increase endogenous GH and result in decreased VAT among HIV-infected patients with fat accumulation, though prior studies have not investigated the interrelationship between augmentation of GH pulsatility and reduced VAT in this population. Taken together, these data suggest that relative GH deficiency is reversible in HIV-infected patients with central fat accumulation, and that this may be achieved with GHRH. This hypothesis will be tested in the current study, as we will determine whether 6 months of treatment with GHRH\textsuperscript{1-44} normalizes GH secretion.

We therefore propose a randomized, placebo-controlled study, in which subjects are randomized to GHRH vs. placebo for 6 months and then all subjects continue on GHRH for an additional 6 month extension period. The study will allow longitudinal analysis of endpoints to assess timing and nature of physiological GH response, e.g., improvement of GH secretion, body composition, insulin sensitivity, adipokine and inflammatory markers, and carotid IMT, as well as the interrelationship between these changes. We will investigate the long-term effects of GHRH\textsuperscript{1-44} on endogenous GH secretion, which will be determined serially with simultaneous assessment of visceral adiposity. Subjects will be randomized to GHRH versus placebo, and endogenous GH pulsatility will be measured at baseline and after 6 months of administration of GHRH\textsuperscript{1-44}. We hypothesize that long-term treatment with GHRH\textsuperscript{1-44} will increase GH pulse secretion. We will investigate the effects of GHRH\textsuperscript{1-44} on insulin sensitivity, other critical fat depots, and adipokine concentrations, again assessing early and late responses. We will also assess important CV endpoints including carotid IMT. Furthermore, we will investigate the contribution of reduced GH secretion to these parameters (IMT, insulin sensitivity, liver/muscle fat, etc.) in regression analyses based on parameters of endogenous GH secretion. In this regard, we hypothesize that IMT and other parameters will be more abnormal among the HIV patients with the most reduced GH secretion.

II. Specific Aims
The specific aims of this study are to determine the effects of 6 months treatment with GHRH\textsuperscript{1-44} vs. placebo in patients with HIV lipodystrophy. In a 12 month study, HIV infected patients with central fat accumulation in the context of treatment for HIV disease will be randomized to 6 months of GHRH\textsuperscript{1-44} vs. placebo in a double-blind fashion and then all subjects continue on GHRH for an additional 6 month extension period. In addition to measurement of the primary endpoint, change in VAT, we will assess:

A. Growth hormone secretion parameters, including GH pulsatility
B. Insulin sensitivity, as assessed by euglycemic, hyperinsulinemic clamp, and ectopic fat accumulation (IMCL and hepatic fat) by MR spectroscopy
C. Cardiovascular indices, including IMT and cardiovascular adipokines, including adiponectin, tPA, CRP and PAI-1, apoproteins and novel inflammatory markers.

III. Subject Selection

60 HIV-infected subjects will be enrolled in this 12 month study. We anticipate that we will need to screen 120 patients in order to randomize 60 patients. During the first 6 months subjects will be randomized to GHRH\textsuperscript{1-44} at 2 mg daily or placebo; following this, all subjects will receive active treatment for an additional 6 months. Subjects will be enrolled based on the following inclusion and exclusion criteria:

**Inclusion Criteria**
1. Men and women age 18-65
2. Previously diagnosed HIV infection
3. Stable antiviral regimen for at least 12 weeks prior to enrollment
4. WC $>95$ cm and WHR $>0.94$ for male, WC $>94$ cm and WHR $>0.88$ for female occurring in the context of treatment for HIV disease
5. Subjective evidence of at least one of the following recent changes, occurring during the treatment of HIV disease: increased abdominal girth, relative loss of fat in the extremities, or relative loss of fat in the face
6. For female subjects, negative mammogram within one year of baseline

**Exclusion Criteria**
1. Use of anti-diabetic agents, Megace, testosterone or any significant steroid use within 6 months of the study. Stable use of testosterone (> 6 mos) at dose equivalent to 200 mg IM q 2 weeks or < 10g/day to skin will be permitted.
2. Use of GH or GHRH within the past 6 months
3. Change in lipid lowering or antihypertensive regimen within 3 months of screening
4. Fasting blood sugar $> 126$ mg/dL, SGOT $> 2.5$ times ULN, Hgb $< 12.0$ g/dL, creatinine $> 1.4$ mg/dL, CD4 count $< 200$
5. Severe chronic illness or active malignancy or history of pituitary malignancy or history of colon cancer
6. For men, history of prostate cancer or evidence of prostate malignancy by PSA $> 5$ ng/mL
7. Prior history of hypopituitarism, head irradiation or any other condition known to affect the GH axis
8. For women, positive urine hCG
9. Oral contraceptives, depo provera or combined progesterone-estrogen injections, transdermal contraceptive patches, estrogen or progestin coated IUD’s within 6 months of the study.
10. Routine MRI exclusion criteria such as the presence of a pacemaker or cerebral aneurysm clip.

IV. Subject Enrollment

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Methods of Enrollment
Preliminary eligibility will be determined based on study staff interviews of interested subjects over the phone. Eligible subjects will then be scheduled for a screening visit.

Informed Consent
Written informed consent will be signed prior to screening evaluation and testing by a licensed physician investigator. Subjects will be informed that they may withdraw from participation in the study at any point.

Randomization
After signing consent and, prior to the baseline visit, eligible subjects will be randomized to receive GHRH<sub>1-44</sub> 2mg SC QD versus identical placebo for 6 months after which all patients will receive active GHRH for an additional 6 months. Randomization will be stratified by gender and testosterone use because of the known effects of gonadal steroids on the GH axis. Randomization will be performed by the MGH Research Pharmacy and will be blinded to study investigators and subjects.

V. Study Procedures
All subject encounters will take place at the MGH GCRC. All female subjects will have a urine pregnancy test at each visit.

Screen Visit (to determine eligibility)
1. Informed consent
2. Detailed medical history and medications (past and current), specifically including antiretroviral medications, antihypertensive and lipid lowering agents and antidiabetic medications
3. Physical exam. For men age 50 or older, this will include a prostate exam.
4. Anthropometric measurements, including waist circumference and waist to hip ratio
5. Fasting blood samples (glucose, creatinine, CBC, liver function tests, CD4 count, and prostate specific antigen for male subjects only and FSH for female subjects only)
6. Urine pregnancy test for all female subjects
7. HIV test and consent

Randomization and Subsequent Visits: Eligible subjects will return for 7 visits. After signing consent and, prior to the baseline visit, subjects will be randomized to receive GHRH<sub>1-44</sub> 2mg SC QD versus identical placebo. During the first 6-month treatment phase, subjects will have visits at baseline, 2 weeks, and months 3, and 6. After month 6, all patients will receive open label GHRH regardless of their original randomization. Subsequent visits will take place at months 6.5, 9 and 12 months. Randomization will be stratified by gender and testosterone use. Subjects and study staff will be blinded to the treatment assignment. In addition, subjects will be randomized to Euglycemic Hyperinsulinemic Clamp subgroup prior to baseline visit based on treatment arm such that 50% of subjects receiving GHRH<sub>1-44</sub> and 50% of subjects receiving placebo will be in this subgroup.

Baseline Visit (start of study) and Month 6 and Month 12
1. Physical exam, medical history and medications (past and current) and smoking history
2. Anthropometrics, including waist and hip circumferences, and resting energy expenditure
3. Overnight frequent blood sampling for GH pulsatility analysis
4. Fasting blood samples for IGF-I, CD4 count, viral load, CRP, adiponectin, tPA, PAI-1, HgbA1c, triglyceride, direct LDL, HDL, cholesterol, lipoprotein (a), Apoprotein B, Apoprotein A1, LDL particle size, LP-PLA2
5. Oral Glucose Tolerance Test
6. MR spectroscopy for IMCL and liver fat content
7. Carotid IMT
8. Whole body, DEXA scan (total lean mass, total fat mass, trunk fat mass and bone density)
9. Single-slice abdominal CT to determine visceral and subcutaneous fat area
10. Dietary analysis from 4 day food record
11. Quality of Life Questionnaire and Modifiable Activity Questionnaire
12. Injection teaching by qualified nursing staff of the GCRC
13. Dispensing study drug and medication diary
14. In addition, subjects in the Clamp subgroup will receive a Euglycemic Hyperinsulinemic Clamp on the morning of Day 2

Interval Assessment 2 weeks and 6.5 weeks (safety and tolerability assessment)
1. Physical exam and interval medical history
2. Fasting blood samples for glucose and IGF-I
3. Injection teaching reinforcement by qualified nursing staff of the GCRC
4. Dispensing study drug and medication diary

Interval Assessment (Month 3 and 9)
1. Physical exam and interval medical history
2. Anthropometrics, including waist and hip circumferences
3. OGTT and euglycemic clamp if assigned to the clamp subgroup
4. Fasting blood samples for glucose and IGF-I
5. Study drug dispensing and medication diary review

Safety Guidelines: Subjects will be discontinued for FBG > 150 mg/dL. Increased FBG will be verified on a subsequent day to insure validity of the value (e.g., to rule out nonfasting or other errors). Similar safety guidelines were used in 2 prior studies of GHRH without adverse effects and with good safety profiles. Subjects will keep a log of injection site reactions including rash. Any subject with injection site rash will be discontinued from the study. Subjects will also be discontinued for any symptoms of GH excess felt to be related to the study drug or for increases in liver or kidney function, or decrease hemoglobin beyond the inclusion and exclusion criteria. Subjects will also be discontinued for use of concomitant medications as outlined in the exclusion criteria. Subjects will keep a log of date and time of injections, adverse events and new concomitant medications.

Study Drug and Dosing: The dose of GHRH\textsuperscript{1-44} will be 2 mg SC QD (TH9507, Theratechnologies, Montreal Canada). Dosing is based on prior studies of this compound in HIV-infected patients with fat accumulation in which GHRH\textsuperscript{1-44} at 2mg significantly reduced
visceral fat of 15%, improved lipid parameters, while achieving a generally physiologic increase in IGF-I of 80% vs. placebo over 6 months\textsuperscript{55}. No clinically apparent effects on insulin and glucose were seen.

**Methods**

**Study Drug**
The dosing for GHRH\textsuperscript{1-44} is 2 mg SQ QD. GHRH\textsuperscript{1-44} and identical-appearing placebo will be dispensed in two 1 mg vials at each study visit. Subjects will be instructed to refrigerate the vials between 36-46° F. An IND for the use of GHRH\textsuperscript{1-44} in HIV lipodystrophy has been obtained: IND 77,473, S. Grinspoon. GHRH\textsuperscript{1-44} (Tesamorelin) has been approved by the FDA for use in HIV lipodystrophy.

**GH Pulsatility Analysis:** Starting at 2000, fasting blood will be sampled every 20 minutes until 0800 for GH levels. Approximately 1.5cc of blood will be drawn at each time point for a total of 55.5cc. Mean overnight GH concentration, basal concentration, pulse frequency, pulse amplitude and overall pattern regularity will be determined using deconvolution analysis with AutoDecon, which employs automated deconvolution algorithms\textsuperscript{56}.

**Single-slice Abdominal Computed Tomography (CT):** Assessment of visceral and subcutaneous abdominal fat will be performed by single-slice CT imaging of the abdomen as previously described\textsuperscript{57} at the level of L4 pedicle which will serve as the landmark for the single slice image. Scan parameters for each image will be standardized (144 table height, 80kV, 70 mA, 2 seconds, 1 cm slice thickness, 48 FOV).

**Carotid Intimal Medial Thickness** will be acquired and analyzed according to a standard protocol at Boston Heart Foundation developed in collaboration with Dr. Chris O’Donnell, Associate Director of the NHLBI Framingham Heart Study, and a recognized expert in IMT assessment\textsuperscript{58-61}. Similar techniques used in the Framingham study will be applied to the patient population to be studied in the proposed grant. We have worked closely with the Boston Heart Association to develop a reliable and accurate protocol for detection of carotid IMT. We have published several papers assessing carotid IMT using this protocol\textsuperscript{62,63}. Carotid Intimal Medial Thickness will be acquired and images will be analyzed according to a standard protocol developed in collaboration with Dr. Linda Hemphill, a recognized expert in IMT assessment. Imaging will be performed with an HDI 5000 Sonos CT machine, using a high-resolution 5-12 MHz linear array transducer. The far wall of the distal one centimeter of the common carotid artery will be imaged at approximately 90 degrees. The precise optimal angle for the demonstration of the area of interest will be noted on Meijer’s carotid arc and a hard copy image will also be stored to facilitate angle reproduction at follow up. This optimal view of the distal one centimeter of common carotid artery is captured digitally and analyzed off line to determine the maximum and mean intimal medial thickness. Images are captured to a Windows NT workstation using a high-quality, high-speed frame capture card made by Data Translation (Marlboro, MA). Imaging of the right carotid is performed with the subject turning his/her head to 225 degrees on Meijer’s arc with the transducer held at 135 degrees. Imaging of the left carotid is performed with the subject turning his/her head to 135 degrees with the transducer held at 225 degrees. 50 frame video clips of this region are acquired onto the Windows NT imaging workstation. Analysis is performed.
with a proprietary edge detection algorithm with published reproducibility. Differences in diameter of the carotid across the 50 frames are used to judge the cardiac cycle and select a frame of minimum diameter (diastole) as our analysis frame. IMT images will be analyzed by a single experienced technician for the entire duration of the study to eliminate temporal drift. A side by side methodology will be utilized for analysis of follow up images vs baseline to further optimize reproducibility. Randomization will be blinded to the reviewer. Every attempt will be made to minimize inter-rater variability. Dr. Hemphill has recently completed IMT measurements in 101 patients using the same technique as will be used in our study. Test-retest variability was low (r=0.93, \(P<0.0001\)) between repeated measures. The difference between scans was 0.0026mm equal to 0.5%.

**Hyperinsulinemic Euglycemic Clamp**: In a subgroup of 30 subjects, insulin sensitivity will be determined using hyperinsulinemic euglycemic clamp. Testing will be completed after a 12-h overnight fast. Patients will receive a primed infusion of regular insulin, 80 mU/m\(^2\) per minute (Humulin, Eli Lilly), for 2 hours. A variable rate of dextrose is administered to maintain blood glucose levels at 5.0 mmol/L (90 mg/dL). Blood samples will be collected for blood glucose determinations before insulin infusion and every 5 min during the 2-hour clamp. Samples for insulin levels will be obtained at 20 min intervals. Insulin sensitivity is assessed as the glucose disposal rate (mg/kg of lean body mass per min), and the ratio of the glucose disposal rate to serum insulin level at steady state will be obtained during the final 20 minutes of the clamp.

**MR Spectroscopy (\(^1\)H MRS) for IMCL and Hepatic Fat Content Measurements**: Imaging procedures will be performed using a 3.0T MR device (Siemens, Erlangen, Germany). Magnetic field strengths of 3.0T are approved by the FDA for clinical use. No intravenous contrast will be used. Total time in MR machine will be approximately 1 hour. \(^1\)H MRS of calf muscles will be performed for determination of lipid concentrations in skeletal muscle at baseline and months 6 and 12. \(^1\)H MRS of tibialis anterior and soleus muscles will be performed after 8-hour overnight fasting. Subjects are positioned feet first in the magnet bore and the right or left calf is placed in a standard radiofrequency transmit and signal receive quadrature extremity coil. A tri-plane gradient echo localizer pulse sequence with echo time (TE) of 1.6 ms and repetition time (TR) of 54.0 ms and axial T1-weighted images (TR, 700 ms; TE, 14 ms; slice thickness, 4 mm; inter-slice gap, 1 mm) of the calf is performed for voxel placement. \(^1\)H MRS data will be acquired using spectroscopic pulse sequences (PRESS, 2D, CSI) with TR of 25 ms, TR of 3,000 ms, 32 acquisitions. In all cases, a voxel is placed on the largest cross-sectional area of the muscle, avoiding visible interstitial tissue, fat or vessels. To ensure consistent positioning in follow-up examination, the axial slice used for voxel placement (counted from proximal fibular tip) is screen-captured with voxel overlays and x-y coordinates. Fitting of all \(^1\)H MRS data is performed using dedicated spectral analysis software, such as LCModel (version 6.0-2, Stephen Provencher, Oakville, Ontario, Canada), and metabolite quantification is performed using eddy current correction and water scaling to yield estimates of intra- and extramyocellular lipids. Liver \(^1\)H MRS will be acquired in all subjects in the supine position and employ a torso coil. We will use spectroscopic pulse sequences (STEAM, PRESS) with parameters such as TR of 3,000 ms, TE of 30 ms, 2,500-Hz sweep width, 2,048 data points, and 8 acquisitions. A localization voxel of 20 mm\(^3\) is placed in the liver with care taken to avoid the large intrahepatic vessels. The selected volume is placed in a similar manner in all the subjects to cover similar regions of
interest. Hepatic lipid concentrations will be obtained using the LCModel quantification software.

**Whole Body DEXA** will be performed using a Hologic Discovery A densitometer to determine total body and regional percent fat and lean body mass. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass\(^6^4\). Trunk, extremity and trunk to extremity ratio will also be assessed\(^6^5,^6^6\).

Biochemical Parameters IGF-I, CD4 count, viral load, CRP, adiponectin, tPA, PAI-1, HgbA1c, triglyceride, direct LDL, HDL, cholesterol, lipoprotein (a), Apoprotein B, Apoprotein A1, LDL particle size, LP-PLA2 will be performed using standard methodologies. AntiTH9507 antibodies will be performed by Theratechnologies.

**Nutritional Analysis** Protein, carbohydrate, fat, micronutrient, dietary supplements and alcohol intake will be determined from 4-day food records (Nutrition Data Systems).

**Anthropometric Measurements**: Measurements of waist to hip ratio, leg circumference, arm circumference, shoulders, back of neck, and neck circumference will be performed using a standardized technique\(^6^7\).

**Indirect Calorimetry**: REE will be determined for a period of 15-30 minutes by indirect calorimetry. A calibrated calorimeter (VMAX29N, Sensormedics) will be used for analysis of VO\(_2\) and VCO\(_2\).

**Questionnaires**: The Phase V questionnaire for Quality of Life for HIV-infected Adults with Lipodystrophy will be used\(^6^8\). The Modifiable Activity Questionnaire will be used to assess activity levels.

**VI. Biostatistical Analysis**

**Sample Size Calculation**

With 60 patients entering the study and taking into account a 20% drop out rate for a total of 48 evaluable patients, the probability is 80% that we can detect a treatment difference of 16.5 (% decrease in VAT by CT) at a two sided \(P=0.05\) percent significance level the GHRH\(^1-44\) treated and placebo-treated patients. This is based on the assumption that the standard deviation of the response variable is approximately 20% based on the Phase III data investigating GHRH\(^1-44\) vs. placebo over 6 months. For secondary endpoints, including and insulin sensitivity, IMCL, hepatic fat and adipocytokine concentrations and IMT, we will be able to detect a clinically relevant 0.83 SD change between treatment groups, with 48 evaluable patients, 80% power, two sided \(\alpha=0.05\).

**Data Interpretation**

The following approach describes the analysis of the prospective observations from the proposed design. Baseline variables will be compared by t-test for continuous variables and chi-
square test for non-continuous variables. The data will be analyzed on an intent-to-treat basis and the analysis will include all available data, including data available from the 3 months time point for certain variables. In the primary analysis, we will use repeated measures ANCOVA to determine the differences between the treatment groups over 6 months, controlling for any variables that are different between the groups at baseline.

Secondary analyses will control for changes in visceral fat, to determine whether reductions in visceral fat contribute independently to the anticipated changes in GH responsivity and insulin sensitivity. We will additionally control for changes in lipid levels in response to GHRH1-44, to determine whether improvement in dyslipidemia contributes independently and/or together with changes in visceral adiposity, to the anticipated changes in insulin sensitivity, and IMT. Finally we will assess whether changes in IGF-I or GH pulsatility (mean GH level, or pulse area) are most related to changes in clinical endpoints fat distribution, insulin sensitivity and adipokine levels and IMT. We hypothesize that changes in GH pulse parameters, more than IGF-I, will be more strongly associated with the observed changes. Diet and activity will be assessed and investigated as potential covariates in the model. We will also determine if responses in primary and secondary endpoints to GHRH1-44 vs. placebo are affected by baseline pulse secretion.

Data from the single cross-over extension phase will be used to determine descriptive statistics, e.g., change from baseline and P value associated for the group receiving GHRH for one year and also for safety data.

VII. Risks and Discomforts

Radiation
As a result of participation in this study, subjects will be exposed to 3 DEXA scans and 3 CT scans over 12 months. The radiation risk associated with the whole body DEXA scan is approximately 1 mrem of radiation exposure, a total of 3 mrem over the entire study. The radiation risk associated with the single slice CT scan of the abdomen is approximately 55 mrem, a total of 165 mrem over the entire study. The total radiation exposure associated with this study is that of 168 mrem. This does not pose excessive risk to subjects.

Mammogram
Women over the age of 40 will be required to have a mammogram if not done within 1 year of study start. The amount of radiation is the same that would be received during a regular annual mammogram.

Blood Drawing
Including frequent sampling, approximately 700cc (1 ½ pints or 3 cups) will be drawn over 12 months. Subjects in the clamp arm will have a total of about 900cc (2 pints or 4 cups) drawn over 12 months. This quantity of blood drawn does not pose excessive risk to patients. Subjects with hemoglobin levels < 12 g/dL will be excluded from the study. The risk of blood loss has been carefully considered and the amount of blood drawn is within the guidelines established by the Human Studies Committee of the MGH. The risks of these procedures are minor bruising or bleeding at the site of the blood draw or IV catheter.
Medications
Administration of all medications will be conducted by the appropriate health care professional. A nurse will be in attendance at all times throughout the inpatient study protocol. A physician will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns.

GHRH\textsuperscript{1-44}: In a combined analysis of two recently completed studies, 806 HIV positive subjects with HIV lipodystrophy (abnormal fat distribution) received either GHRH\textsuperscript{1-44} 2 mg once daily or placebo for 6 months. The study showed three results with respect to adverse events among subjects taking GHRH\textsuperscript{1-44}: 1). There was no significant difference in serious adverse events between subjects on placebo and GHRH\textsuperscript{1-44}. 2). The adverse events listed below were experienced by more than 10% of the study participants. However, the percentage of subjects with any of these adverse events did not significantly differ between subjects on placebo and GHRH\textsuperscript{1-44}:

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>GHRH\textsuperscript{1-44} (% Patients)</th>
<th>Placebo (% Patients)</th>
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<tbody>
<tr>
<td>Joint Pain</td>
<td>13.3 %</td>
<td>11.0%</td>
</tr>
<tr>
<td>Headache</td>
<td>10.9%</td>
<td>11.0%</td>
</tr>
<tr>
<td>Injection Site Bruising</td>
<td>7.4%</td>
<td>10.3%</td>
</tr>
</tbody>
</table>

3. The adverse events listed below were experienced by more than 5% but less than 10% of the study participants receiving GHRH\textsuperscript{1-44} and were different compared to placebo. There were no other differences in any other events reported by subjects in both groups:

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>GHRH\textsuperscript{1-44} (% Patients)</th>
<th>Placebo (% Patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Site Redness</td>
<td>8.5%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Injection Site Itchiness</td>
<td>7.6%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>6.1%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Myalgias</td>
<td>5.5%</td>
<td>1.9%</td>
</tr>
</tbody>
</table>

Clinically significant changes were not observed for liver function (alanine transaminase), kidney function (creatinine), or blood pressure (diastolic and systolic). The majority of the reported side effects were mild in severity. In addition, skin reactions suggestive of hypersensitivity developed at the site of injection and beyond in a small percentage (2.9%) of subjects treated with GHRH\textsuperscript{1-44}. These subjects were discontinued and did well. Some of these subjects tested positive for antibodies against GHRH\textsuperscript{1-44} and native growth hormone releasing hormone, but there was no relationship between the development of antibodies and changes in IGF-I or VAT. The antibodies were non neutralizing and decreased with discontinuation of GHRH. The significance of these antibodies is unknown.

Insulin: Administration of insulin infusion can cause hypoglycemia.
Other Procedures: Indirect calorimetry, carotid ultrasound and MR spectroscopy have no known health risks.

Other Risks: It is possible that incidental abnormal findings may be found during this study. In this case, the subject and his/her primary care physician will be notified.

VIII. Potential Benefits
It is hoped that the treatment will improve visceral fat, fat redistribution, insulin sensitivity, lipids, and adipokine and potentially IMT by increasing GH secretion. Information obtained from this study will provide insight into the use of GHRH1-44 in patients with HIV lipodystrophy. Future studies will build upon the data obtained in this study to allow for the further development of GHRH1-44 as a treatment strategy in this population. Therefore, the benefits of this study are felt to outweigh its risks. As GHRH has been approved by the FDA, all subjects will receive at least 6 months of GHRH in the current protocol. The current study investigates critical parameters and endpoints not previously investigated in prior studies.

IX. Monitory and Quality Assurance
The study investigators will monitor all data collected for this study. Data will be stored in locked Program in Nutritional Metabolism offices.

An independent data and safety monitoring board will be established including an endocrinologist, HIV expert and community advocate. The DSMB will meet every 3 months to review safety data and adverse events. The study will also be monitored continuously by the principal investigator of the study. Subjects will be discontinued with any of the following: hemoglobin value <11 g/L; AST or ALT value >3x upper limit normal; creatinine value >1.5 mg/dL; for verified (repeated once for confirmation) blood glucose >150 mg/dL. Lab abnormalities will be verified on a subsequent day to insure validity of the value (e.g. to rule out nonfasting or other errors). Similar safety guidelines were used in 2 prior studies of GHRH without adverse effects and with good safety profiles. Subjects will keep a log of injection site reactions including rash. Any subject with injection site rash will be discontinued from the study. Subjects will also be discontinued for any symptoms of GH excess felt to be related to the study drug or for increases in liver or kidney function, or decrease hemoglobin beyond the inclusion and exclusion criteria. Subjects will also be discontinued for use of concomitant medications as outlined in the exclusion criteria. Subjects will keep a log of date and time of injections, adverse events and new concomitant medications.

Subjects will be instructed to report immediately significant redness and rash occurring beyond the injection site. Any systemic symptoms that are observed along with significant skin reactions beyond the injection site should be promptly reported. These systemic symptoms include, but are not limited to nausea, shortness of breath, abundant sweating and tachycardia. These symptoms will be carefully monitored and subjects with extended rash or systemic symptoms will be discontinued. A physician will be available on-call 24 hours/day, 7 days/week, to all study participants for any questions or concerns.

Adverse events will be reported to the Subcommittee on Human Subjects at Massachusetts General Hospital in accordance with adverse event reporting guidelines, and all serious adverse
events will be reported in less than 24 hours. Adverse events will also be reported to the FDA in accordance with FDA reporting guidelines. The safety contact for this study is Dr. Steven Grinspoon.

X. References


