Original Investigation

Effect of Tesamorelin on Visceral Fat and Liver Fat in HIV-Infected Patients With Abdominal Fat Accumulation
A Randomized Clinical Trial

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IMPORTANCE Among patients infected with human immunodeficiency virus (HIV), visceral adiposity is associated with metabolic dysregulation and ectopic fat accumulation. Tesamorelin, a growth hormone–releasing hormone analog, specifically targets visceral fat reduction but its effects on liver fat are unknown.

OBJECTIVE To investigate the effect of tesamorelin on visceral and liver fat.

DESIGN, SETTING, AND PATIENTS Double-blind, randomized, placebo-controlled trial conducted among 50 antiretroviral-treated HIV-infected men and women with abdominal fat accumulation at Massachusetts General Hospital in Boston. The first patient was enrolled on January 10, 2011; for the final patient, the 6-month study visit was completed on September 6, 2013.

INTERVENTIONS Participants were randomized to receive tesamorelin, 2 mg (n=28), or placebo (n=22), subcutaneously daily for 6 months.

MAIN OUTCOMES AND MEASURES Primary end points were changes in visceral adipose tissue and liver fat. Secondary end points included glucose levels and other metabolic end points.

RESULTS Forty-eight patients received treatment with study drug. Tesamorelin significantly reduced visceral adipose tissue (mean change, −34 cm^2 [95% CI, −53 to −15 cm^2] with tesamorelin vs 8 cm^2 [95% CI, −14 to 30 cm^2] with placebo; treatment effect, −42 cm^2 [95% CI, −71 to −14 cm^2]; $P = .005$) and liver fat (median change in lipid to water percentage, −2.0% [interquartile range {IQR}, −6.4% to 0.1%] with tesamorelin vs 0.9% [IQR, −0.6% to 3.7%] with placebo; $P = .003$) over 6 months, for a net treatment effect of −2.9% in lipid to water percentage. Fasting glucose increased in the tesamorelin group at 2 weeks (mean change, 9 mg/dL [95% CI, 5-13 mg/dL] vs 2 mg/dL [95% CI, −3 to 8 mg/dL] in the placebo group; treatment effect, 7 mg/dL [95% CI, 1-14 mg/dL]; $P = .03$), but changes at 6 months in fasting glucose (mean change, 4 mg/dL [95% CI, −2 to 10 mg/dL] with tesamorelin vs 2 mg/dL [95% CI, −4 to 7 mg/dL] with placebo; treatment effect, 2 mg/dL [95% CI, −6 to 10 mg/dL]; $P = .72$ overall across time points) and 2-hour glucose (mean change, −1 mg/dL [95% CI, −18 to 15 mg/dL] vs −8 mg/dL [95% CI, −24 to 8 mg/dL], respectively; treatment effect, 7 mg/dL [95% CI, −16 to 29 mg/dL]; $P = .53$ overall across time points) were not significant.

CONCLUSIONS AND RELEVANCE In this preliminary study of HIV-infected patients with abdominal fat accumulation, tesamorelin administered for 6 months was associated with reductions in visceral fat and additionally with modest reductions in liver fat. Further studies are needed to determine the clinical importance and long-term consequences of these findings.

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In human immunodeficiency virus (HIV) infection, visceral adipose tissue accumulation is associated with ectopic fat accumulation in the liver.[1-3] Patients infected with HIV demonstrate a high prevalence of nonalcoholic fatty liver disease (NAFLD), estimated at 30% to 40%,[1,2,4] which is seen often in the context of increased visceral adipose tissue.[1,2] Nonalcoholic fatty liver disease encompasses simple steatosis, characterized by triglyceride accumulation in hepatocytes ("liver fat"), as well as steatohepatitis, characterized by inflammation, hepatocellular injury, and fibrosis that may progress to end-stage liver disease and hepatocellular carcinoma. To date, there are no approved pharmacologic strategies to reduce liver fat, and no strategies have proven successful in HIV-infected patients. A substudy of HIV-infected individuals participating in a trial of growth hormone and rosiglitazone[5] showed no change in liver fat with rosiglitazone and a trend for reduction in liver fat with growth hormone.[6]

ART *antiretroviral therapy*

HOMA-IR *homeostasis model assessment of insulin resistance*

IGF-1 *insulinlike growth factor 1*

NAFLD *nonalcoholic fatty liver disease*

The current study investigates changes in liver fat using a different treatment approach, in which a growth hormone–releasing hormone analog, tesamorelin, is administered to increase endogenous pulsatile growth hormone. Tesamorelin reduces visceral adipose tissue with minimal effects on subcutaneous fat,[7,8] but its effects on other ectopic fat depots and detailed metabolic indexes have not been investigated.

Methods

Patient Selection

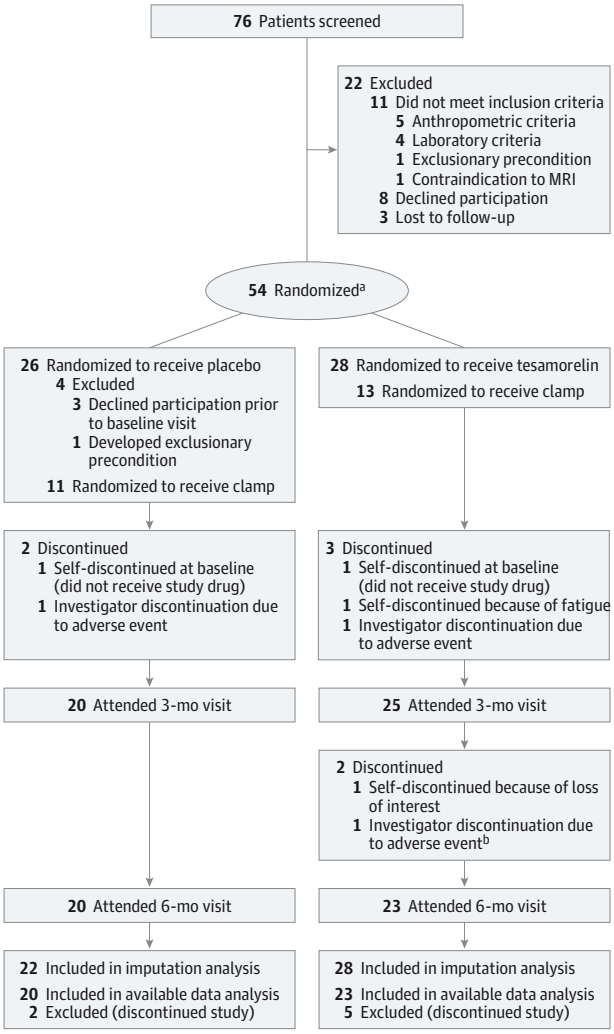
Potential participants were identified through referral from infectious disease physicians, advertisements in community centers and health clinics, and the clinical research study volunteer program. Patients underwent screening, and eligible patients were invited to participate. Fifty men and women with HIV infection and increased abdominal adiposity participated in a baseline assessment. Recruitment began in December 2010. The first patient was enrolled on January 10, 2011, and the final study visit was completed on September 6, 2013. The study was approved by the Massachusetts General Hospital Institutional Review Board, and written informed consent was obtained from each patient prior to study procedures (see study protocol in Supplement 1).

Patients with HIV infection aged 18 to 65 years with stable use of antiretroviral therapy (ART) for 3 months or longer who noted body fat changes including abdominal fat accumulation in the context of ART and who had objective evidence of abdominal adiposity as determined by sex-specific criteria (waist circumference ≥95 cm for men and ≥94 cm for women; waist-to-hip ratio ≥0.94 for men and ≥0.88 for women[9]) were included. Patients with a history of pituitary disease or cranial irradiation, use of growth hormone or growth hormone–releasing hormone during the past 6 months, or use of supraphysiologic corticosteroids, gonadal steroids except physiologic testosterone replacement, or antidiabetic agents were excluded. Lipid-lowering and antihypertensive medications were allowed if doses were stable for 3 months or more prior to baseline. Patients were excluded for pregnancy, inability to undergo magnetic resonance imaging, severe chronic illness, any active malignancy, and history of colon cancer, prostate cancer, or pituitary malignancy. Laboratory exclusion criteria were fasting glucose greater than 126 mg/dL, aspartate aminotransferase greater than 2.5 times the upper limit of normal, hemoglobin less than 12 g/dL, creatinine greater than 1.4 mg/dL, CD4 cell count less than 200/mL, and, for men, prostate-specific antigen greater than 5 ng/mL. Patients with increased prostate-specific antigen were excluded to avoid enrolling patients with abnormal prostate growth. Three patients had participated in previous randomized trials of tesamorelin in our research group,[7,10,11] but, per protocol, none of these individuals had received tesamorelin in the 6 months prior to enrollment.

Study Design

After screening, eligible volunteers underwent 2 independent randomizations, a double-blind 1:1 randomization to tesamorelin, 2 mg/d subcutaneously, vs identical placebo (**Figure 1**) and, independently, a 1:1 randomization to undergo euglycemic hyperinsulinemic clamp in addition to other study procedures. Randomization was stratified by sex and, for men, by physiologic testosterone use using a permuted-block algorithm within each stratum, with randomly varying block sizes of 2, 4, or 8. Baseline assessment included fasting blood sampling for lipids, insulinlike growth factor 1 (IGF-1), complete blood cell count, CD4 cell count, HIV viral load, hemoglobin A_{1c}, C-reactive protein, adiponectin, aspartate aminotransferase, and alanine aminotransferase; 75-g oral glucose tolerance test; waist and hip circumferences; dual-energy x-ray absorptiometry (Hologic, Discovery A) for total body and regional fat mass; single-slice computed tomography at L4 for assessment of visceral and subcutaneous adipose tissue area[12,13]; hydrogen 1 (^{1}H) magnetic resonance spectroscopy for hepatocellular lipid to water percentage and intramyocellular lipid of the tibialis anterior and soleus muscles[14,15]; overnight frequent sampling for growth hormone concentrations; and neck ultrasound for measurement of carotid intima-media thickness.[16] ^{1}H magnetic resonance spectroscopy was performed in the morning following an 8-hour fast. Two patients did not follow instructions to fast for their 6-month scans. According to the intention-to-treat design of the study, data from these patients were retained in the analyses; changes in liver fat remained significant between groups in sensitivity analyses excluding these patients (see Results section). All images were performed on the same scanner. Calculation of liver fat from spectroscopy data was automated, and results were reviewed by a single radiologist, blinded to treatment assignment, to ensure quality control. With regard to reproducibility, Bland-Altman analysis of scans repeated using our technique showed a mean difference between same-day scans of 0.29% (95% CI, −1.46% to 2.05%).[14] The diagnostic accuracy of ^{1}H magnetic resonance spectroscopy for liver steatosis is high, with an area under the receiver operating characteristic curve of 0.94 (95% CI, 0.88-1.0) compared with

Figure. Participant Flow in the Study of Tesamorelin for Visceral and Liver Fat in HIV



Two statistical analyses were performed using (1) an imputation approach to handle missing data and (2) all available data, with missing data treated as missing.

[a] Two randomization events, a double-blind 1:1 randomization to tesamorelin vs placebo and a 1:1 randomization to euglycemic hyperinsulinemic clamp, occurred simultaneously prior to the baseline visit. These randomization events were independent of each other.

[b] Follow-up magnetic resonance spectroscopy and computed tomography data obtained at 3-mo visit for this participant.

assessment of liver biopsy by an experienced pathologist.[17] For measurement of visceral and subcutaneous adipose tissue, single-slice computed tomography has an estimated correlation between repeat measurements of 0.99, with errors in precision estimated at 1.9% for subcutaneous adipose tissue and 3.9% for visceral adipose tissue.[18] Dietary intake, including alcohol, was assessed by 4-day food record (Nutrition Data System). Physical activity was assessed using the Modifiable Activity Questionnaire.[19] For assessment of overnight growth hormone, patients had dinner at 5 PM and began fasting at 6 PM. Blood samples were drawn every 20 minutes from 8 PM until 7:40 AM. At the conclusion of the baseline assessment, patients received their first dose of study drug, which they administered daily for the next 6 months. Patients returned for a safety visit 2 weeks after baseline, a 3-month assessment including oral glucose tolerance test, and a 6-month assessment identical to baseline. Patients randomized to the euglycemic hyperinsulinemic clamp subset (n = 13 in the tesamorelin group and n = 11 in the placebo group) also underwent clamp procedure at baseline, 3 months, and 6 months (eAppendix in Supplement 2). Full clamp data were not available for 3 patients in the tesamorelin group and 2 patients in the placebo group. Adherence to the study medication was measured by patient-completed study diary and by vial count of returned study drug. Data on self-reported race and ethnicity were collected as these characteristics may affect fat distribution.

Laboratory Methods

Growth hormone (Beckman Access Ultrasensitive Assay), insulin (Beckman Access), total adiponectin (Alpco), and high-sensitivity C-reactive protein (Labcorp) were measured by immunoassays. Insulinlike growth factor 1 was measured by liquid chromatography/mass spectroscopy (Quest Diagnostics). Lipids, glucose, and transaminases were measured by standard clinical assays (Labcorp). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated.[20]

Statistical Analysis

Given the absence of prior data on hepatic fat, the study was powered for visceral adipose tissue reduction, with the hypothesis that tesamorelin would reduce visceral fat in the abdomen and related ectopic depots. The protocol was therefore initially designed with visceral adipose tissue as the primary end point, but prior to trial initiation, because of increasing interest in liver fat as a critical end point, we reconsidered the end points and made hepatic fat a co-primary end point, with secondary end points including intramyocellular lipid, measures of glucose homeostasis, lipid, carotid intima-media thickness, transaminases, and systemic inflammatory markers as listed in the initial ClinicalTrials.gov posting dated December 15, 2010, prior to enrollment of the first patient. The protocol was initially planned to enroll 60 patients, with an estimated 48 planned to complete the study, providing 80% power to detect a treatment effect of 16.5% change in visceral adipose tissue. Because of issues with drug supply, recruitment stopped a few months earlier than anticipated, resulting in 43 patients completing the study. Based on this change in enrollment and more recent data regarding the standard deviation of change in visceral adipose tissue with tesamorelin (41 cm^2) from the combined phase 3 studies,[8] post hoc power calculations showed that the sample size of 43 patients had 85% power to detect a treatment difference of 38.5 cm^2 in change in visceral adipose tissue at a 2-sided $\alpha = .05$.

Data were tested for normality using the Shapiro-Wilk test. Normally distributed variables are presented as means with standard deviations or, for changes over time, as means with 95% confidence intervals; variables that are not normally distributed are presented as medians with interquartile ranges

(IQRs). At baseline, comparisons between treatment groups for categorical variables were made using the Pearson χ^2. For continuous variables, comparisons were made using the *t* test for normally distributed variables or the Wilcoxon rank sum test for variables that were not normally distributed.

Analysis for treatment effect was based on a modified intention-to-treat population among patients with available baseline and 6-month follow-up data. For variables measured only at baseline and 6 months, including the primary end points of visceral adipose tissue and hepatic fat, between-group comparisons of changes over time were made using the *t* test for normally distributed variables or the Wilcoxon rank sum test for non-normally distributed variables. For hepatic fat, data were missing at baseline for 1 patient in the tesamorelin group and at follow-up for 8 patients (3 in the placebo group and 5 in the tesamorelin group). For visceral adipose tissue, follow-up data were missing in 6 patients (2 in the placebo group and 4 in the tesamorelin group). Sample sizes for each analysis are provided in each table. To handle missing data, analyses using an imputation approach confirmed the results of the analyses using all available data for hepatic fat and visceral adipose tissue, as well as for secondary end points assessed at baseline and 6 months (eTable 1 in Supplement 2). An additional analysis was performed using logistic regression to assess the significance of treatment group in predicting liver fat reduction controlling for age, duration of HIV infection, and lipid-lowering therapy. Secondarily, within-group comparisons were made using the paired *t* test for normally distributed variables and the Wilcoxon signed rank test for non-normally distributed variables.

For outcomes measured at more than 2 time points (eg, baseline, 3, and 6 months), random intercept mixed-effects modeling using restricted maximum likelihood was applied to assess the significance of the time × randomization interaction. Two analyses were performed: a mixed-effects analysis using all available data and a mixed-effects analysis performed to handle missing data using imputation for missing values (eTable 1 in Supplement 2).

Treatment effect and 95% confidence interval are shown for normally distributed data. For non-normally distributed data, statistical determination of a treatment effect and associated 95% confidence interval is not possible, but an approximate net treatment effect was determined by subtracting the median changes in each group. Changes within each group for non-normally distributed data are presented showing the median and IQR of the paired changes over time in each group, whereas the data presented at each time point represent the median and IQR at such points for each group. Subtraction of the group medians may differ from the medians of the paired changes because of normality of data.

Relationships between continuous variables were assessed using the Pearson correlation coefficient (denoted as *r*) when both variables were normally distributed and the Spearman rank correlation coefficient (denoted as ρ) when one or both variables was not normally distributed. For comparisons of interest (eg, change in visceral adipose tissue by change in liver fat), we performed multivariable linear regression modeling, including treatment group and a group × x-variable interaction term, to assess whether associations were different between treatment groups.

P values shown in the text for aggregate changes over time between groups for primary and secondary end points are those for imputation analyses. In tables, *P* values from both imputation and from analysis using all available data are shown. All statistical analyses were 2-sided, with $\alpha = .05$ as the predefined threshold for statistical significance. Data analysis was performed with SAS, version 9.3, and JMP, version 10.0.0 (SAS Institute Inc).

Results

Of 76 patients who completed eligibility screening, 50 were randomized and underwent baseline assessment (Figure). Reasons for patient exclusion are listed in the Figure. Two patients participated in the baseline visit but discontinued before starting study drug. Patient disposition during the study is shown in the Figure. Median overall adherence by vial count was 98% (IQR, 87%-100%) in the tesamorelin group and 99% (IQR, 88%-99%) in the placebo group (*P* = .95). Adherence by study diary was similar: median, 99% (IQR, 97%-100%) in the tesamorelin group and 99% (IQR, 97%-100%) in the placebo group (*P* = .51). One patient in the placebo group and 2 patients in the tesamorelin group had adherence of less than 80% (*P* = .65).

Baseline Characteristics

There were no differences between treatment groups in baseline demographics, alcohol use, or hepatitis C status (**Table 1**). No patient reported consuming alcohol equivalent to 3 or more drinks per day. Menopausal status did not differ (75% postmenopausal in both groups; *P*>.99). Duration of HIV, antiretroviral therapy use, and lipid-lowering therapy use did not differ at baseline (Table 1). Body composition did not differ at baseline (**Table 2**), nor were there differences between groups in measures of glucose homeostasis (**Table 3**); lipids, transaminases, or inflammatory markers (eTable 2 in Supplement 2); immunologic measures (Table 2); or dietary intake and activity (eTable 3 in Supplement 2).

Baseline measures of visceral fat and liver fat were positively associated (ρ = 0.42; *P* = .003), and both showed associations with measures of glucose homeostasis and lipids (eTable 4 in Supplement 2). Both visceral adipose tissue (ρ = -0.43; *P* = .003) and liver fat (ρ = -0.44; *P* = .003) were negatively associated with baseline overnight mean growth hormone concentrations and showed no association with baseline IGF-1.

Changes in Body Composition and Ectopic Fat

The tesamorelin group experienced a significant decrease in mean abdominal visceral adipose tissue area (-34 cm^2; 95% CI, -53 to -15 cm^2 vs placebo, 8 cm^2; 95% CI, -14 to 30 cm^2; treatment effect, -42 cm^2; 95% CI, -71 to -14 cm^2; *P* = .005) without effects on mean subcutaneous adipose tissue area (tesamorelin, 2 cm^2; 95% CI, -5 to 10 cm^2 vs placebo, 8 cm^2; 95% CI, -3 to 20 cm^2; treatment effect, -6 cm^2; 95% CI, -19 to

Table 1. Baseline Characteristics

Characteristics	Tesamorelin (n = 28)	Placebo (n = 22)
Age, median (IQR), y	49 (46-54)	53 (49-58)
Sex, No. male/ No. female (% male)	24/4 (86)	18/4 (82)
Race/ethnicity, No. (%)[a]		
White	20 (71)	14 (64)
Black	6 (21)	3 (14)
Hispanic	1 (4)	3 (14)
Other	1 (4)	2 (9)
Smoking status, No. (%)		
Never	14 (50)	9 (41)
Past	9 (32)	10 (45)
Current	5 (18)	3 (14)
Alcohol use, median (IQR), g/d	0 (0-6)	0 (0-0.3)
Duration of HIV, mean (SD), y	17 (7)	20 (6)
Hepatitis C, No. (%)	7 (25)	5 (23)
Medication use at baseline, No. (%)		
NRTIs	28 (100)	21 (95)
NNRTIs	14 (50)	15 (68)
PIs	11 (39)	10 (45)
Other antiretroviral therapy[b]	7 (25)	6 (27)
Lipid-lowering therapy	13 (46)	13 (59)
Statin	8 (29)	11 (50)
Testosterone	7 (25)	6 (27)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitors.

[a] Collected via patient self-report.

[b] Other antiretroviral therapy including entry inhibitors and integrase inhibitors.

7 cm^2; P = .29) (Table 2). Mean change in visceral adipose tissue was -9.9% (95% CI, -19.7% to -0.2%) with tesamorelin vs 6.6% (95% CI, -4.1% to 17.3%] with placebo, for a net treatment effect of -16.6% (95% CI, -30.6% to -2.6%), similar to that seen in previous studies.[7,8] Hepatic lipid to water percentage decreased significantly in the tesamorelin group (median, -2.0%; IQR, -6.4% to 0.1%) compared with placebo (median, 0.9%; IQR, -0.6% to 3.7%; P = .003), for a net effect between groups of −2.9% in lipid to water percentage (Table 2). This effect of tesamorelin on liver fat remained statistically significant (P = .005) controlling for age, duration of HIV, and lipid-lowering therapy. In a sensitivity analysis excluding 2 patients who were not fasting for ^{1}H magnetic resonance spectroscopy, both in the placebo group, the change in liver fat remained significant (P<.001). For the 3 patients with poor adherence, change in hepatic fat was within the IQR for the respective treatment groups. Both total fat and trunk fat as measured by dual-energy x-ray absorptiometry decreased significantly compared with placebo (Table 2). Intramyocellular lipid did not change (Table 2).

Changes in Glucose Homeostasis

Fasting glucose increased in the tesamorelin group compared with the placebo group between baseline and 2 weeks (mean change: tesamorelin, 9 mg/dL; 95% CI, 5-13 mg/dL vs placebo, 2 mg/dL; 95% CI, -3 to 8 mg/dL; treatment effect, 7 mg/dL; 95% CI, 1-14 mg/dL; P = .03 at 2 weeks) (Table 3) but was not different from baseline at subsequent assessments (mean change at 3 months: tesamorelin, 6 mg/dL; 95% CI, 2-10 mg/dL vs placebo, 2 mg/dL; 95% CI, -4 to 7 mg/dL; treatment effect, 4 mg/dL; 95% CI, -2 to 11 mg/dL; P = .20 at 3 months; mean change at 6 months: tesamorelin, 4 mg/dL; 95% CI, -2 to 10 mg/dL vs placebo, 2 mg/dL; 95% CI, -4 to 7 mg/dL; treatment effect, 2 mg/dL; 95% CI, -6 to 10 mg/dL; P = .56 at 6 months) (Table 3). Mixed-effects modeling showed no significant effects of tesamorelin on fasting glucose (P = .72 overall across time points), fasting insulin (P = .68), or HOMA-IR (P = .45) (Table 3) over the 6-month period. There was a slight but statistically significant increase in hemoglobin A_{1c} from baseline to 6 months (mean change: tesamorelin, 0.20%; 95% CI, 0.04%-0.36% vs placebo, 0.02%; 95% CI, -0.07% to 0.10%; treatment effect, 0.19%; 95% CI, 0.01%-0.36%; P = .03). One patient in each treatment group progressed from impaired fasting glucose to diabetes by fasting glucose measurement, whereas 1 additional patient in each group progressed from impaired glucose tolerance to diabetes by 2-hour oral glucose tolerance test (see eTable 5 in Supplement 2 for distribution of glucose values). During the 6-month treatment period, no patient in either group experienced fasting blood glucose levels greater than 150 mg/dL, which was the predetermined cutoff for study discontinuation.

In the euglycemic hyperinsulinemic clamp subgroup, there was a significant difference in the change from baseline to 3 months in insulin-stimulated glucose uptake, whereby insulin sensitivity decreased in the tesamorelin group and increased in the placebo group (mean change: tesamorelin, -0.5 mg/kg/min; 95% CI -1.7 to 0.7 mg/kg/min vs placebo, 1.3 mg/kg/min; 95% CI, 0.6-2.1 mg/kg/min; treatment effect, -1.8 mg/kg/min; 95% CI, -3.3 to -0.4 mg/kg/min; P = .02). In contrast, the change from baseline was not significant at 6 months (mean change: tesamorelin, 0.4 mg/kg/min; 95% CI, -1.2 to 1.9 mg/kg/min vs placebo, 0.7 mg/kg/min; 95% CI, -0.6 to 2.1 mg/kg/min; treatment effect, -0.4 mg/kg/min; 95% CI, -2.3 to 1.5; P = .68). Results were similar when insulin-stimulated glucose uptake was corrected for steady-state insulin level and, at 6 months, for lean body mass.

Changes in Transaminases

There were no significant overall changes in alanine aminotransferase, whereas aspartate aminotransferase decreased with tesamorelin (median change, −4 U/L; IQR, -12 to 2 U/L) compared with placebo (median change, 0 U/L; IQR, -6 to 5 U/L; P = .046) (eTable 2 in Supplement 2).

Changes in Cardiovascular Risk Measures

Intima-media thickness of the left carotid artery decreased in the tesamorelin group (mean change, -0.03 mm; 95% CI, -0.07 to -0.00 mm; P = .04) but did not change in the placebo group (mean change, -0.00 mm; 95% CI, -0.03 to 0.03 mm; P = .89), though the primary comparison between groups was not significant (treatment effect, -0.03 mm; 95% CI, -0.08 to 0.01 mm; P = .14) (Table 2). Blood pressure and lipids did not significantly change (eTable 2 in Supplement 2). C-reactive protein

Table 2. Effects of Tesamorelin on Body Composition, Ectopic Fat, Carotid Intima-Media Thickness, and Immunologic Measures

	Baseline[a]		6 mo		Change After 6 mo[b]		Treatment Effect, All Available Data[c]	*P* Value	
	Tesamorelin (n = 28)	Placebo (n = 22)	Tesamorelin (n = 23)	Placebo (n = 20)	Tesamorelin	Placebo		All Available Data[c]	Imputation[d] (Range of Estimates)
Body composition									
Visceral adipose tissue, mean (SD), cm^2	208 (98) (n = 28)	237 (127) (n = 22)	165 (59) (n = 24)[e]	252 (131) (n = 20)	-34 (-53 to -15)[f] (n = 24)	8 (-14 to 30) (n = 20)	-42 (-71 to -14)	.005	.005 (-54 to -35)
Subcutaneous adipose tissue, mean (SD), cm^2	258 (116) (n = 28)	256 (123) (n = 21)	247 (121) (n = 24)[e]	272 (149) (n = 20)	2 (-5 to 10) (n = 24)	8 (-3 to 20) (n = 19)	-6 (-19 to 7)	.37	.29 (-14 to -1)
Body mass index, median (IQR)[g]	28.1 (25.8-32.7) (n = 28)	30.1 (27.0-33.2) (n = 22)	28.5 (25.4-30.7) (n = 23)	30.3 (27.2-35.1) (n = 20)	0.3 (-0.3 to 0.8) (n = 23)	0.3 (-0.2 to 0.8) (n = 20)	0.0	.89	.62
Lean mass, mean (SD), kg	60.7 (10.1) (n = 28)	62.4 (9.7) (n = 22)	61.5 (9.3) (n = 23)	62.6 (9.8) (n = 20)	0.4 (-0.8 to 1.5) (n = 23)	-0.5 (-1.6 to 0.5) (n = 20)	0.9 (-0.6 to 2.4)	.23	.21 (0.4 to 1.6)
Fat mass, median (IQR), kg	24.4 (20.3-30.6) (n = 28)	26.4 (22.4-33.3) (n = 22)	23.3 (19.8-30.9) (n = 23)	27.2 (24.4-33.8) (n = 20)	-0.2 (-1.6 to 1.4) (n = 23)	1.2 (0.3 to 3.4)[f] (n = 20)	-1.4	.04	.02
Trunk fat, median (IQR), kg	13.9 (11.1-16.8) (n = 28)	15.4 (13.8-18.1) (n = 22)	12.6 (11.0-16.7) (n = 23)	15.9 (15.2-18.2) (n = 20)	-0.4 (-1.4 to 0.7) (n = 23)	0.6 (0.1 to 1.7)[f] (n = 20)	-1.0	.01	.004
Ectopic fat									
Liver fat, hepatocellular lipid-to-water %, median (IQR)	4.5 (2.0-19.3) (n = 27)[h]	6.2 (2.1-20.6) (n = 22)	4.2 (1.8-11.2) (n = 23)[e]	7.2 (4.6-19.8) (n = 19)	-2.0 (-6.4 to 0.1)[f] (n = 22)	0.9 (-0.6 to 3.7) (n = 19)	-2.9	.004	.003
Soleus IMCL/Cr, median (IQR)	13.7 (7.1-18.6) (n = 28)	16.1 (8.5-27.7) (n = 22)	10.6 (7.7-14.8) (n = 23)[e]	11.4 (7.0-24.8) (n = 20)	-1.7 (-3.9 to 0.7) (n = 23)	-0.2 (-5.2 to 5.5) (n = 20)	-1.5	.46	.19
Tibialis IMCL/Cr, median (IQR)	4.3 (2.8-5.8) (n = 28)	3.3 (2.5-5.8) (n = 22)	3.9 (2.3-5.7) (n = 24)[e]	4.1 (2.9-6.0) (n = 20)	-0.3 (-2.1 to 0.8) (n = 24)	0.2 (-2.3 to 2.0) (n = 20)	-0.5	.39	.39
Carotid intima-media thickness, mean (SD), mm	0.76 (0.16) (n = 27)	0.84 (0.18) (n = 22)	0.71 (0.15) (n = 23)	0.82 (0.20) (n = 20)	-0.03 (-0.07 to -0.00)[f] (n = 23)	-0.00 (-0.03 to 0.03) (n = 20)	-0.03 (-0.08 to 0.01)	.15	.14 (-0.05 to -0.02)
Immunologic/ virologic measures									
CD4 cells, mean (SD), %	33 (10) (n = 28)	31 (10) (n = 22)	33 (9) (n = 22)	32 (11) (n = 20)	0 (-1 to 2) (n = 22)	1 (0 to 3)[f] (n = 20)	-1 (-3 to 1)	.26	.32 (-2 to 0)
CD8 cells, mean (SD), %	43 (12) (n = 28)	46 (10) (n = 22)	42 (11) (n = 22)	46 (9) (n = 20)	-1 (-2 to 0) (n = 22)	-1 (-2 to 0)[f] (n = 20)	0 (-1 to 2)	.62	.73 (-1 to 1)
Viral load, median (IQR), log_{10} copies/mL	0 (0-1.7) (n = 28)	0 (0-1.7) (n = 22)	0 (0-1.4) (n = 22)	0 (0-1.0) (n = 20)	0 (-1.0 to 0.0) (n = 22)	0 (-0.5 to 0) (n = 20)	0	.97	.51

Abbreviations: IMCL/Cr, intramyocellular lipid-to-creatinine ratio; IQR, interquartile range.

[a] Between-group testing for comparison of baseline values used the *t* test for normally distributed data and Wilcoxon rank sum for non–normally distributed data. No statistically significant differences were seen between groups at baseline. Sample size for each variable at each time point is given.

[b] Normally distributed data are presented as mean (95% CI) for change after 6 months; data that are not normally distributed are presented as median (IQR) for change after 6 months. Within-group testing used the paired *t* test for normally distributed data and the Wilcoxon signed rank test for non–normally distributed data.

[c] Treatment effect and *P* value are for a modified intention-to-treat analysis using all available data. The *t* test was used to compare changes between groups for normally distributed end points and the Wilcoxon rank sum test was used to compare changes between groups for end points that were not normally distributed. Treatment effect is shown as mean (95% CI) for normally distributed end points and as net difference between median changes in each group for end points that were not normally distributed.

[d] *P* values for imputation analyses: for normally distributed end points, multiple imputation was performed by replacing missing values with imputed values calculated over 100 iterations, using longitudinal mixed-effects modeling, and discarding the first 10 iterations. The *P* value is the average of the *P* values from the individual runs of the multiply imputed data sets. The values in parentheses provide a range (2.5th percentile to 97.5th percentile) of the estimated effect sizes for the imputation analyses. For non–normally distributed end points, imputation analysis was performed by replacing the missing data for the 0- to 6-mo changes using the median of the change in the combined groups. The *P* value given is for the Wilcoxon rank sum test for comparison of change between groups using the imputed data set. For these data, range of estimates is not available.

[e] Magnetic resonance imaging and computed tomography data from 1 patient who was discontinued between the 3 and 6 mo visits for adverse event are included. These data were obtained at a termination visit.

[f] Significant within-group difference between baseline and 6 months ($P < .05$).

[g] Calculated as weight in kilograms divided by height in meters squared.

[h] Data on hepatic fat from 1 patient were excluded because of a technical problem with scan acquisition.

Table 3. Glucose Homeostasis

	Baseline[a]		2 wk		3 mo		6 mo		Change After 6 mo[b]		Treatment Effect, All Available Data[c]	*P* Value	
	Tesamorelin	Placebo	Tesamorelin	Placebo	Tesamorelin	Placebo	Tesamorelin	Placebo	Tesamorelin	Placebo		All Available Data[c]	Imputation (Range of Estimates)[d]
Fasting glucose, mean (SD), mg/dL	87 (8) (n = 28)	91 (13) (n = 22)	97 (12)[e] (n = 26)	92 (11) (n = 21)	94 (9) (n = 25)	92 (10) (n = 20)	91 (13) (n = 23)	92 (16) (n = 20)	4 (-2 to 10) (n = 23)	2 (-4 to 7) (n = 20)	0 (-6 to 6)	.94	.72 (-3 to 3)
2-h glucose, mean (SD), mg/dL	114 (31) (n = 28)	130 (29) (n = 22)	NA	NA	119 (43) (n = 25)	125 (29) (n = 20)	118 (44) (n = 22)	123 (36) (n = 18)	-1 (-18 to 15) (n = 22)	-8 (-24 to 8) (n = 18)	8 (-11 to 27)	.43	.53 (-6 to 18)
Fasting insulin, median (IQR), μIU/mL	7.9 (4.7-11.1) (n = 28)	8.0 (3.9-16.8) (n = 22)	NA	NA	8.1 (4.7-12.8) (n = 25)	10.8 (6.8-13.7) (n = 20)	7.9 (4.8-11.3) (n = 22)	7.6 (4.5-11.7) (n = 20)	-0.2 (-2.6 to 1.8) (n = 22)	0.5 (-4.9 to 3.0) (n = 20)	0.6 (-3.5 to 4.8)	.76	.68 (-0.7 to 2.3)
HOMA-IR, median (IQR)	1.6 (1.1-2.5) (n = 28)	1.9 (0.9-3.6) (n = 22)	NA	NA	1.8 (1.0-3.0) (n = 25)	2.6 (1.5-2.9) (n = 20)	1.7 (1.0-2.5) (n = 22)	1.7 (1.1-3.0) (n = 20)	0.0 (-0.8 to 0.5) (n = 22)	0.2 (-1.1 to 0.8) (n = 20)	0.4 (-0.7 to 1.4)	.48	.45 (0.0 to 0.8)

SI conversion factors: To convert glucose to mmol/L, multiply by 0.0555. To convert insulin to pmol/L, multiply by 6.945.

Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; NA, data not available (not measured).

[a] No statistically significant differences between groups at baseline.

[b] Normally distributed data are presented as mean (95% CI) for change after 6 months; data that are not normally distributed are presented as median (IQR) for change after 6 months.

[c] Treatment effect (95% CI) and *P* value are for a mixed-effects model (time × randomization) using all available data over 6 months.

[d] *P* values for imputation analyses: multiple imputation was performed by replacing missing values with imputed values calculated over 100 iterations, using longitudinal mixed-effects modeling, and discarding the first 10 iterations. The *P* value is the average of the *P* values from the individual runs of the multiply imputed data sets. The values in parentheses provide a range (2.5th percentile to 97.5th percentile) of the estimated effect sizes for the imputation analyses.

[e] Significant change from baseline in the tesamorelin vs placebo groups ($P < .05$). Change from baseline at each time point was assessed using the *t* test.

did not significantly change, whereas tesamorelin tended to increase adiponectin ($P = .07$) (eTable 2 in Supplement 2).

Changes in Growth Hormone and IGF-1

Changes from baseline in IGF-1 and IGF-1 *z* scores were significantly different between treatment groups at 2 weeks, 3 months, and 6 months of treatment (eTable 6 in Supplement 2). Mean overnight growth hormone also increased significantly in the tesamorelin group (median change, 0.35 ng/mL; IQR, 0.15-0.57 ng/mL) compared with placebo (median change, -0.01; IQR, -0.07 to 0.06 ng/mL; $P<.001$). eFigure 1 in Supplement 2 shows the median and IQR of growth hormone at each overnight sampling time point.

Nutrition and Physical Activity

There were no significant changes in dietary intake or physical activity (eTable 3 in Supplement 2). Alcohol intake also did not significantly change over 6 months (median change: tesamorelin, 0 g/d; IQR, 0-4 g/d vs placebo, 0 g/d; IQR, 0-0 g/d; $P = .79$).

Interrelationship of Reductions in Ectopic Fat With Metabolic Changes and Glucose

Among all patients, changes in hepatic lipid were significantly associated with changes in visceral adipose tissue ($\rho = 0.31$; $P = .047$) (eFigure 2 in Supplement 2), HOMA-IR ($\rho = 0.50$; $P = .001$), and fasting insulin ($\rho = 0.50$; $P = .001$). See eTable 7 in Supplement 2 for correlations with change in liver fat by treatment group.

Change in visceral adipose tissue was significantly associated with change in mean growth hormone ($\rho = -0.46$; $P = .005$), whereas change in hepatocellular lipid to water percentage was not associated with change in mean growth hormone ($\rho = -0.22$; $P = .21$).

Safety and Adverse Events

Adverse events that occurred in greater than 5% of patients are reported in **Table 4**. There were 3 serious adverse events in both the treatment and placebo groups. Serious adverse events in the tesamorelin group consisted of 1 hospitalization due to exacerbation of existing congestive heart failure, 1 hospitalization for pneumonia, and 1 diagnosis of basal cell carcinoma in a patient with a history of the same. Serious adverse events in the placebo group consisted of 1 hospitalization for acute stroke, 1 hospitalization for Heller myotomy, and 1 diagnosis of basal cell carcinoma in a patient with a history of the same. Two patients underwent blinded dose reductions (eAppendix and eTable 6 in Supplement 2). For further information on adverse events, see Table 4 and the eAppendix in Supplement 2.

There were no significant changes in immunologic measures in the tesamorelin group (Table 2).

Discussion

In this preliminary study, our data demonstrate a modest but statistically significant decrease in liver fat with tesamorelin

Table 4. Adverse Events

Events	No. (%) Tesamorelin	No. (%) Placebo	*P* Value
Any adverse event	25 (89)	21 (95)	.42
Events resulting in discontinuation from study[a]	3 (11)	1 (5)	.42
Serious adverse events	3 (11)	3 (14)	.75
Hospitalization for CHF exacerbation	1	0	
Hospitalization for pneumonia	1	0	
Basal cell carcinoma	1	1	
Hospitalization for Heller myotomy	0	1	
Hospitalization for acute stroke	0	1	
Adverse events occurring in >5% of patients			
Injection site bruising	10 (36)	11 (50)	.31
Paresthesia	6 (21)	1 (5)	.09
Injection site erythema	4 (14)	2 (9)	.57
Arthralgia	4 (14)	4 (18)	.71
Injection site stinging	3 (11)	0	.11
Myalgia	3 (11)	0	.11
Hyperglycemia[b]	2 (7)	2 (9)	.80
Edema	2 (7)	1 (5)	.70
Sinusitis	2 (7)	1 (5)	.70
Dose adjustment[c]	2 (7)	0	.20

Abbreviation: CHF, congestive heart failure.

[a] Reasons for study discontinuation in the tesamorelin group were as follows: (1) investigator discontinuation due to CHF exacerbation, (2) investigator discontinuation due to basal cell carcinoma, and (3) self-discontinuation due to fatigue. The reason for study discontinuation in the placebo group was investigator discontinuation due to stroke.

[b] Hyperglycemia was defined as fasting glucose >126 mg/dL or glucose >200 mg/dL on 2-h oral glucose tolerance test at any visit.

[c] Institutional review board–approved dose reductions to 1 mg/d were performed for 2 patients because of paresthesia. Study blinding was maintained.

in HIV-infected individuals selected for abdominal fat accumulation, although the clinical importance of this finding is uncertain. Liver fat and visceral fat were closely associated at baseline, and the reduction in liver fat during the study was significantly associated with the reduction in visceral adipose tissue.

To our knowledge, the data from this study are the first to demonstrate in a clinical trial that an agent selectively reducing visceral fat simultaneously reduced liver fat independent of changes in weight. Thus, our data support the hypothesis that visceral fat accumulation is linked to liver fat accumulation and suggest that selective targeting of visceral adipose tissue reduction can lead to reductions in liver fat. The mechanisms by which growth hormone augmentation reduced liver fat are unknown. Growth hormone augmentation by tesamorelin may increase oxidation of visceral fat. In addition, growth hormone may reduce liver fat through inhibition of hepatic de novo lipogenesis[21,22] or other mechanisms. Two prior articles investigated growth hormone replacement in non-HIV hypopituitary models and showed mixed results on hepatic fat.[23,24] In contrast, the current study used growth hormone-releasing hormone to augment endogenous growth hormone secretion as a strategy to reduce visceral fat in an HIV model selected for excess visceral adipose tissue.

The decrease in liver fat in this study suggests that strategies to reduce visceral adiposity merit further investigation in HIV-infected patients with NAFLD, a condition for which there are no approved treatments. Importantly, NAFLD is associated with visceral adiposity and other metabolic abnormalities in HIV.[1,25] Although the causal pathways underlying these interrelationships are not yet clear, visceral adiposity results in increased inflammatory cytokine production and increased portal free fatty acid flux, either or both of which may contribute to steatohepatosis and hepatic insulin resistance.[26-28]

In this study, tesamorelin resulted in reductions in visceral adipose tissue without reductions in subcutaneous adipose tissue. Subcutaneous fat is thought to represent a beneficial depot that may serve as a buffer to protect against ectopic fat distribution into other organs.[26,29-31] Strategies such as tesamorelin, which are selective to visceral adipose tissue and do not simultaneously reduce subcutaneous adipose tissue, may be optimal to reduce ectopic fat. Further studies of the effects of tesamorelin on other depots linked to visceral adipose tissue, including epicardial fat, should be performed in HIV-infected patients.

Our data also further elucidate effects of tesamorelin on glucose homeostasis. Administration of growth hormone increases glucose.[13,32] In contrast, studies to date have suggested that tesamorelin has limited adverse effect on glucose homeostasis.[7,8,33] Our data demonstrate that tesamorelin initially perturbed glucose as well as insulin sensitivity as assessed by clamp. However, these initial changes were reversed and glucose returned to baseline over longer durations of treatment. We showed a modest increase in hemoglobin A_{1c}, consistent with data from larger studies of tesamorelin,[7,8] which may reflect initial increases in glucose.

Our study has limitations. First, the purpose of this study was to determine detailed metabolic end points, including those involving ^{1}H magnetic resonance spectroscopy and euglycemic hyperinsulinemic clamp measurements, limiting sample size. Thus, the study may have been underpowered to detect changes in secondary end points. Nonetheless, nonsignificant improvement in adiponectin and significant

improvements in aspartate aminotransferase suggest additional metabolic effects of visceral adipose tissue reduction in the HIV-infected population. In this study, we chose to enroll patients based on the Food and Drug Administration–approved indication for tesamorelin to reduce abdominal fat, and we determined benefits to liver fat and metabolic indexes. Because the cohort was not specifically chosen for increased liver fat and the absolute change in lipid to water percentage was modest, the clinical significance of our data are not known. Changes in liver fat may have been more pronounced in a cohort specifically selected for NAFLD. Nonalcoholic fatty liver disease may have a benign clinical course and may not progress to liver disease. Liver biopsies, which are the gold standard for assessing features of steatohepatitis and advanced liver disease, were not performed in this study. Our population was primarily male and had been living with HIV and receiving ART for a long period, consistent with many patients exhibiting lipodystrophic changes in fat. Although abdominal hypertrophy may be less common with newer ART, there exists a substantial group of patients with abdominal fat accumulation in the context of long-term prior ART. Furthermore, we did not collect data following discontinuation of tesamorelin. Previous studies have shown that visceral fat may reaccumulate after discontinuation of tesamorelin,[34] and future studies will be necessary to determine if reductions in liver fat with tesamorelin are maintained following treatment discontinuation. Moreover, tesamorelin is expensive, which is a barrier to its use.

Conclusions

In this preliminary study of HIV-infected patients with abdominal fat accumulation, tesamorelin administered for 6 months was associated with reductions in visceral fat and additionally with modest reductions in liver fat. Further studies are needed to determine the clinical importance and long-term consequences of these findings.

ARTICLE INFORMATION

Author Contributions: Dr Grinspoon had full access to all of the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Stanley, Lee, Grinspoon.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Stanley, Feldpausch, Oh, Lee, Torriani, Grinspoon.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Stanley, Feldpausch, Lee, Grinspoon.
Obtained funding: Grinspoon.
Administrative, technical, or material support: Stanley, Feldpausch, Oh, Branch, Torriani.
Study supervision: Grinspoon.

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