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Effects of Tesamorelin on Inflammatory Markers in HIV Patients with Excess Abdominal Fat: Relationship with Visceral Adipose Reduction

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Abstract

Objective—To report the effects of tesamorelin, a growth hormone releasing hormone analogue, on inflammatory and fibrinolytic markers and to relate these effects to changes in visceral adipose tissue (VAT).

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Design and Methods—410 HIV-infected patients with abdominal adiposity were randomized to 2 mg tesamorelin (n=273) or placebo (n=137) subcutaneously daily for 26 weeks. Circulating PAI-1 antigen, tPA antigen, CRP, and adiponectin were assessed.

Results—At baseline, VAT was significantly associated with PAI-1 antigen ($\rho = 0.36$, $P < 0.001$), tPA antigen ($\rho = 0.29$, $P < 0.001$), CRP ($\rho = 0.18$, $P < 0.001$), and adiponectin ($\rho = -0.22$, $P < 0.001$). Treatment with tesamorelin resulted in a significant decrease from baseline in tPA antigen (-2.2 ± 2.5 vs. -1.6 ± 2.9 ng/mL, tesamorelin vs. placebo, $P < 0.05$). Changes in PAI-1 antigen were not significant in the tesamorelin group compared to placebo. Among patients receiving tesamorelin, changes in inflammatory markers were associated with change in VAT (PAI-1 antigen: $\rho = 0.16$, $P = 0.02$; tPA antigen: $\rho = 0.16$, $P = 0.02$; adiponectin: $\rho = -0.27$, $P < 0.001$), and these associations remained significant when controlling for changes in IGF-1.

Conclusion—In HIV patients with abdominal adiposity, tesamorelin may have a modest beneficial effect on adiponectin and fibrinolytic markers in association with changes in VAT. Further studies are needed to determine the clinical significance of these changes. These data further highlight the deleterious role of excessive VAT and the utility of strategies to improve VAT in this population.

Keywords

tesamorelin; GHRH; HIV; intra-abdominal fat; adiponectin; tissue plasminogen activator; plasminogen activator inhibitor 1

Introduction

A significant percentage of HIV-infected patients develop increased visceral adipose tissue (VAT) accumulation [1], and this is seen most clearly in studies in which antiretroviral naïve patients begin HAART [2]. Increased visceral and upper trunk fat are associated with dyslipidemia [3], insulin resistance [4], decreased circulating adiponectin [5, 6], increased rates of subclinical atherosclerosis [7], and elevated risk of cardiovascular disease (CVD) [8]. Abdominal fat accumulation may also be associated with increased CRP [9], which is a strong predictor of CVD in HIV-infected individuals [10]. Moreover, individuals with HIV-associated visceral fat accumulation may have impaired fibrinolysis. The hemostatic markers plasminogen activator inhibitor-1 (PAI-1) antigen and tissue plasminogen activator (tPA) antigen are increased in HIV-infected individuals with abdominal obesity compared to controls [5, 11], and both PAI-1 antigen and tPA antigen are strongly associated with cardiovascular risk in the general population [12–14].

We have previously reported that treatment with a novel GHRH analogue, tesamorelin, for 26 weeks decreases visceral adiposity and increases adiponectin in HIV-infected individuals with abdominal fat accumulation, without significant effects on CRP [15]. As tesamorelin was recently approved by the United States Food and Drug Administration, further information regarding its effects on inflammatory adipokines and hemostatic markers is needed. We hypothesized that the reductions in VAT achieved with tesamorelin would be associated with improvement in circulating hemostatic markers tPA antigen and PAI-1 antigen. Using data from the previously described cohort, we sought to determine the effect of tesamorelin on PAI-1 and tPA antigens in HIV-infected individuals with abdominal adiposity. Moreover, we sought to investigate if changes in inflammatory markers were related to reductions in VAT controlling for augmentation in GH, as estimated by changes in IGF-I. Previous reports have indicated that GH treatment decreases tPA antigen in non-HIV infected individuals [16, 17], but it has been unclear whether this is a direct effect of GH itself or whether decreased tPA antigen may be mediated by decreased abdominal adiposity. In the current report, we show that long-term treatment with tesamorelin decreases tPA

antigen without significantly decreasing PAI-1 antigen, and that changes in tPA, as well as the previously reported increase in adiponectin, appear to be related to reductions in visceral adiposity but not to changes in IGF-I.

Subjects and Methods

Subjects

Four hundred and ten HIV-infected patients (352 males and 58 females, age range: 28–65 years) were recruited at 43 sites between June 2005 and April 2006 to participate in a 26-week placebo-controlled Phase 3 trial to assess the efficacy and safety of tesamorelin in reducing VAT [15]. Patients were randomly assigned in a ratio of 2:1 to receive either 2 mg of tesamorelin or matching placebo, administered by subcutaneous injection, for 26 weeks. At 26 weeks, patients initially in the tesamorelin group were re-randomized to either continue tesamorelin (T-T group, N = 154) or receive placebo (T-P group, N = 50) from weeks 26–52. Body composition and metabolic endpoints from this extension phase, including information on CRP and adiponectin, but not tPA antigen or PAI-1 antigen, were previously published [18].

The primary endpoint of the study was the percent change from baseline to Week 26 in VAT. Eligibility criteria included the receipt of stable ART for at least 8 weeks and presence of abdominal fat accumulation, defined as a waist circumference ≥ 95 cm and waist-to-hip ratio ≥ 0.94 for males and a waist circumference ≥ 94 cm and waist-to-hip ratio ≥ 0.88 for females (for complete eligibility criteria, see Falutz et al. [15]). Waist circumference and waist-to-hip ratio criteria were based on cutoffs that define increased visceral adiposity (VAT > 130 cm²) in men and women in the general population [19]. The study was approved by the Institutional Review Board at each site, and participants provided written informed consent.

Laboratory Methods

VAT was determined by computerized tomography (CT) scan from a single 5-mm slice obtained at the level of L4 and L5 intervertebral disc [15, 20]. Images were read in a blinded fashion at a central imaging reading center (Perceptive Informatics, Waltham, MA, USA). Inflammatory markers were measured centrally at Gamma-Dynacare (Brampton, Ontario, Canada). Serum PAI-1 antigen and tPA antigen levels were measured from blood samples drawn in CTAD vacutainers (containing sodium citrate, theophylline, adenosine and dipyridamole) using ELISA (enzyme-linked immunosorbent assay) kits (Biopool, Umea, Sweden). The intra-assay coefficient of variation (CV) for the PAI-1 antigen assay is 3.6–16.9%, and the inter-assay CV is 4.3–6.8%. The intra-assay coefficient of variation (CV) for the tPA antigen assay is 7.8–9.5%, and the inter-assay CV is 4.6–9.7%. CRP was measured by nephelometry using a BNII analyzer (Siemens Diagnostics) with a mean CV of 6.4%. Serum adiponectin levels were determined using an ELISA kit (B-Bridge International, Inc., Sunnyvale, CA, USA); intra-assay CV is 3.0–5.3%, and inter-assay CV is 6.3–7.6%. IGF-1 was measured at Esoterix (Calabasas Hills, CA, USA).

Statistical Analysis

Baseline comparisons between groups were made using an analysis of variance (ANOVA) for continuous variables and Fisher's exact test for noncontinuous variables. The treatment effects were determined using an analysis of covariance (ANCOVA) accounting for baseline and randomization effects as previously described [15]. These analyses were done on the intent-to-treat (ITT) population, which was defined as all randomized subjects who were exposed to study drug (i.e. injection of at least one dose of study drug), with the last-observation-carried-forward for imputation of missing data. To assess the effects of

continued tesamorelin or withdrawal from weeks 26–52 on tPA and PAI-1, within group comparisons were performed using repeated measures ANOVA. Spearman's correlations were performed to evaluate the relationships between baseline values and changes from baseline to Week 26 in inflammatory markers as well as the relationships between inflammatory markers and VAT or IGF-I, using the ITT population. Partial correlations were used to examine relationships between VAT and inflammatory markers controlling for age, gender, race, BMI, and IGF-I. Partial correlations were also performed to determine the association between changes in inflammatory parameters and changes in VAT after adjustment for change in IGF-I, and separately to determine the association between changes in inflammatory parameters and changes in IGF-I, after adjustment for changes in VAT. Mean $\pm$ SD are reported unless otherwise indicated. $P < 0.05$ was considered significant in all analyses.

Results

Patients

The baseline characteristics of patients enrolled into the study are shown in Table 1. Body composition, metabolic, inflammatory and fibrinolytic parameters were similar between the study groups at baseline (all $P > 0.05$). Mean waist circumference was 104 ± 10 cm, and mean waist-to-hip ratio was 1.05 ± 0.06 . Mean CRP was elevated at $>4\text{mg/L}$ in both groups. Mean VAT was $>170\text{cm}^2$ in both groups, far exceeding thresholds that have been associated with increased cardiovascular risk [21–23].

Relationships Between Inflammatory Markers, VAT, and IGF-1 at Baseline

At baseline, VAT was positively associated with PAI-1 ($\rho = 0.36$, $P < 0.001$), tPA ($\rho = 0.29$, $P < 0.001$), and CRP ($\rho = 0.18$, $P < 0.001$) in all patients in univariate modeling. An inverse association was found between VAT and adiponectin ($\rho = -0.22$, $P < 0.001$) (Table 2). When controlling for age, gender, race, and IGF-I in multivariate modeling, VAT remained significantly associated with PAI-1 antigen ($\rho = 0.35$, $P < 0.001$), tPA antigen ($\rho = 0.24$, $P < 0.001$), CRP ($\rho = 0.25$, $P < 0.001$), and, inversely, with adiponectin ($\rho = -0.24$, $P < 0.001$). Results were similar when controlling for age, gender, race, IGF-I and BMI (data not shown).

IGF-I levels showed weak inverse associations with PAI-1 antigen ($\rho = -0.10$, $P = 0.04$) and tPA antigen ($\rho = -0.10$, $P = 0.04$) concentrations, whereas there was no association between IGF-I and adiponectin ($\rho = 0.01$, $P = 0.85$) or CRP ($\rho = -0.10$, $P = 0.09$). In exploratory analyses, a quadratic term was added to the models associating IGF-I with these markers, and there were no significant curvilinear associations with IGF-I (data not shown).

Changes in VAT and IGF-I after 26 Weeks of Tesamorelin Administration

As previously reported [15], VAT significantly decreased from baseline to Week 26 in tesamorelin-treated patients ($-27.8 \pm 38.7 \text{ cm}^2$ (-15%) vs. $5.1 \pm 36.4 \text{ cm}^2$ (5%), tesamorelin vs. placebo, $P < 0.001$ vs. placebo, treatment effect, -20.0%) and IGF-I significantly increased (109 ± 113 vs. $-16 \pm 66 \text{ ng/mL}$, $P < 0.001$ vs. placebo). The change in VAT was significantly negatively associated with the change in IGF-I ($\rho = -0.24$, $P < 0.001$). Effects of tesamorelin on subcutaneous fat and body weight were not seen [15].

Changes in Inflammatory Markers after 26 Weeks of Tesamorelin Administration

Treatment with tesamorelin was associated with a small but significant decrease in tPA antigen (-2.2 ± 2.5 vs. $-1.6 \pm 2.9 \text{ ng/mL}$, tesamorelin vs. placebo, $P = 0.03$) as compared to placebo (Table 3). Change in PAI-1 antigen in the tesamorelin group was not significantly different from placebo (-2.5 ± 16.9 vs. $-1.0 \pm 17.3 \text{ ng/mL}$, tesamorelin vs. placebo, $P =$

0.34, Table 3). As previously reported, adiponectin modestly but significantly increased from baseline in tesamorelin-treated patients (0.5 ± 2.7 vs. -0.1 ± 1.3 $\mu\text{g/mL}$, tesamorelin vs. placebo, respectively, $P = 0.03$), and CRP did not significantly change with tesamorelin treatment (Table 3) [15].

Relationships between Baseline Values and Changes in Inflammatory Markers after 26 weeks of Tesamorelin Administration

The changes in inflammatory markers were related to the degree of baseline elevation of these markers. Among patients receiving tesamorelin, significant and inverse correlations were found between baseline PAI-1 antigen ($\rho = -0.44$, $P < 0.001$), tPA antigen ($\rho = -0.46$, $P < 0.001$), CRP ($\rho = -0.50$, $P < 0.001$), and adiponectin ($\rho = -0.14$, $P = 0.037$) and the change in these variables with 26 weeks of treatment.

Relationships between Changes in Inflammatory Markers and Changes in IGF-1 and VAT after 26 Weeks of Tesamorelin Administration

Within the tesamorelin group, changes in PAI-1 antigen ($\rho = 0.16$, $P = 0.02$) and tPA antigen ($\rho = 0.16$, $P = 0.02$) were significantly and positively associated with the percent change in VAT at Week 26, and change in adiponectin ($\rho = -0.27$, $P < 0.001$) was inversely related to the change in VAT (Table 4). Associations between change in VAT and changes in PAI-1 antigen ($P = 0.03$), tPA antigen ($P = 0.02$), and adiponectin ($P < 0.001$) remained significant when controlling for change in IGF-I, and these relationships also remained significant when using partial correlations controlling for age, race, and gender in addition to change in IGF-1 (data not shown). By contrast, changes in PAI-1 antigen, tPA antigen, and adiponectin were not significantly related to changes in IGF-I, with or without controlling for changes in VAT (Table 4).

Changes in tPA and PAI-1 after Continued Tesamorelin or Withdrawal (26 – 52 weeks)

In the extension dataset, significant changes in tPA antigen seen over the first 26 weeks were sustained during weeks 26–52 in the group that continued tesamorelin (T-T group, change from baseline at 52 weeks -2.66 ± 2.60 ng/mL, $p < 0.001$ for within group comparison). Among the subjects discontinuing tesamorelin during weeks 26–52 (T-P group), the change from baseline at 52 weeks in tPA antigen was -2.19 ± 2.22 ng/mL ($p < 0.001$ for within group comparison). No significant changes were seen in PAI-1 antigen during the extension phase. As previously published, adiponectin showed sustained increase in the T-T group but returned to baseline in the T-P group, and there were no significant changes in CRP during the extension phase [18].

Discussion

The current report demonstrates that treatment with the GHRH analogue tesamorelin decreased tPA antigen concentrations in HIV-infected individuals. There were no significant changes in PAI-1 antigen with tesamorelin as compared to placebo. At baseline, higher tPA antigen, higher PAI-1 antigen, and lower adiponectin were associated with VAT in both univariate and multivariate modeling, consistent with previous reports [24–26]. Changes in PAI-1 and tPA antigens, as well as increased adiponectin [15], were significantly associated with decreases in VAT, even when controlling for changes in IGF-1, a marker for the effects of tesamorelin on the GH axis. By contrast, there were not strong associations between IGF-1 and inflammatory markers at baseline, and changes in PAI-1 antigen, tPA antigen, and adiponectin were not associated with changes in IGF-I. Taken together, these data suggest that the changes in adiponectin and tPA after tesamorelin treatment may not be due to GH augmentation *per se* but are likely due to other factors including the effects of tesamorelin to decrease VAT. In addition, these data demonstrate that in addition to

reducing VAT, tesamorelin may have additional cardiometabolic benefits in this population by reducing tPA antigen and increasing adiponectin. These effects may be useful for the HIV population, in whom myocardial infarction risk is known to be increased compared to non-HIV infected controls [27].

Impaired fibrinolysis may play an important role in the pathophysiology of thrombotic events, and numerous hemostatic markers, including tPA antigen and PAI-1 antigen, are strongly associated with cardiovascular disease risk in the general population [12, 14]. Although tPA is anti-thrombotic, dissolving fibrin in the circulation, assays of tPA antigen primarily measure inactive tPA/PAI-1 complexes in the circulation, such that increased tPA antigen levels indicate decreased fibrinolysis [28]. In the general population, increased tPA antigen independently predicts coronary heart disease in models adjusting for conventional cardiovascular risk factors [29, 30]. In HIV-infected patients, Hadigan et al. demonstrated increased tPA antigen levels in HIV-infected individuals with body fat changes compared to non-HIV infected BMI- and age-matched controls [11]. In the current report, we extend this observation by demonstrating that tPA antigen levels are strongly associated with VAT in this population, even when controlling for age, gender, race, and IGF-1 levels. Furthermore, we show that tesamorelin significantly decreases tPA antigen in association with reductions in VAT. The magnitude of reduction in the tesamorelin group (-2.2ng/mL) is similar to that seen after treatment with metformin in this population [11], although the decrease compared to the placebo group (-2.2 vs. -1.6) is relatively modest and smaller than the difference between metformin and placebo. In contrast to metformin, no effects were seen from tesamorelin on PAI-1. In addition, although it is difficult to directly compare results from this study and the prior unrelated studies of metformin, performed 8 years earlier, fibrinolytic markers appeared less elevated at baseline in the current study. Taken together these data suggest that metformin, an insulin sensitizing agent, more robustly decreases fibrinolytic markers than tesamorelin, which also has beneficial albeit smaller effects. A cost benefit analysis is difficult to perform directly between metformin, which is indicated to improve insulin resistance and is available in generic form, and newly approved tesamorelin, indicated to improve visceral adiposity but not insulin resistance.

Our data demonstrate that favorable changes in tPA antigen are not significantly associated with changes in IGF-I, suggesting that decreased tPA antigen may not be a direct effect of augmenting GH but rather may be mediated, in part, by favourable body composition changes resulting from tesamorelin. We cannot rule out the possibility that other biochemical effects of tesamorelin also contribute to the reduction in tPA antigen. It is unlikely that the changes in tPA and other inflammatory and fibrinolytic markers were due to overall changes in weight or subcutaneous fat, as these body composition parameters did not change in response to tesamorelin, which had a selective effect to reduce VAT. The reductions in tPA antigen seen with tesamorelin treatment persisted to some degree after the switch to placebo during weeks 26–52, despite reaccumulation of VAT in this period, and levels at 52 weeks remained significantly below initial baseline.

PAI-1 antigen levels are strongly associated with visceral adiposity in the general population [26, 31] and are reported to be higher in HIV-infected individuals compared to controls in association with increased visceral adiposity [32] and protease inhibitor use [33]. In keeping with these reports, our data demonstrate a significant relationship between PAI-1 antigen and visceral fat at baseline. However, PAI-1 antigen did not significantly change in the tesamorelin group compared to the placebo group in this study.

We previously reported that tesamorelin treatment significantly increases adiponectin [15], and we now show that changes in adiponectin are also strongly associated with changes in VAT but not IGF-I. Adiponectin is known to be inversely associated with visceral adiposity,

and adiponectin demonstrates a strong inverse association with cardiovascular disease [34–36] in the general population. Further, in animal models, administration of adiponectin demonstrates cardioprotective effects [37, 38]. In HIV-infection, adiponectin is negatively associated with visceral fat and positively associated with limb fat, and adiponectin concentrations are reduced in individuals with lipodystrophic changes compared to HIV-infected controls without body fat changes [39, 40]. In the current study, we demonstrate that a selective reduction in VAT through administration of tesamorelin is significantly associated with improvements in adiponectin in this population. To our knowledge these are among the first data to demonstrate that a pharmacologic therapy specifically targeted to reduce VAT has the associated effect of increasing adiponectin. We cannot be certain, however, that reduction in VAT is the primary factor contributing to improved adiponectin in the context of tesamorelin treatment. Alternatively, decreases in VAT may be associated with reduction in subclinical inflammation or other factors that subsequently contribute to increased adiponectin concentrations. We do not have data on high molecular weight (HMW) adiponectin, and further studies will be needed to characterize the effects of tesamorelin on the ratio of HMW:total adiponectin.

There are important limitations of the current study. First, the study population consists largely of white male participants. Gender and race were controlled for in analyses, but fibrinolytic markers may differ by gender and race [41, 42], and further studies will be needed to determine if the effects of tesamorelin differ according to gender or race. Second, many inflammatory markers show substantial intra-individual variance, which may reduce our ability to characterize changes in these markers over time. In addition, there is a possibility of Type I error. Finally, the changes in inflammatory markers in response to tesamorelin were of relatively small magnitude, and, as the study did not measure clinical cardiovascular endpoints, these changes are of uncertain clinical significance. Moreover, metformin appears to have a larger effect on PAI-1 in this population and may have a more significant effect on tPA [11]. Although tesamorelin should not be used for the primary purpose of improving these markers, modest effects to decrease tPA antigen and increase adiponectin may be relevant associated benefits of its use to reduce VAT.

In summary, our data suggest that tesamorelin decreases tPA antigen and increases adiponectin in association with reductions in VAT. Reductions in tPA persisted after discontinuation of tesamorelin for 6 months, whereas adiponectin levels returned to baseline following withdrawal. Our data do not show a relationship between changes in tPA antigen, PAI-1 antigen, and adiponectin and changes in IGF-I during the study, suggesting that augmentation of GH may not have a direct effect on these variables. Rather, our data suggest that reductions in VAT in response to tesamorelin contribute to improvement in inflammatory and fibrinolytic markers. Of note, however, IGF-I levels do not fully reflect changes in GH dynamics, including alterations in pulsatility, so the current analysis does not completely eliminate the possibility that changes in GH dynamics played some role in changes in inflammatory cytokines and fibrinolytic markers. Recent data demonstrate effects of tesamorelin on GH pulsatility in healthy non-HIV subjects [44], and further studies will be needed to examine relationships between GH pulse characteristics and these variables. Additional studies will also be needed to examine the effects of tesamorelin on other hemostatic markers. These data shed light on the relationship of excess VAT to inflammatory markers in a model of VAT excess in which we show a baseline relationship and a change in response to decreases in VAT. These data also have potential clinical implications and suggest that use of tesamorelin in patients with HIV and excess abdominal fat accumulation may result in an overall improvement in critical inflammatory and fibrinolytic markers, which may improve overall cardiovascular risk.

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References

1. Nguyen A, Calmy A, Schiffer V, et al. Lipodystrophy and weight changes: data from the Swiss HIV Cohort Study, 2000–2006. *HIV Med.* 2008; 9:142–150. [PubMed: 18218001]
2. Shlay JC, Bartsch G, Peng G, et al. Long-term body composition and metabolic changes in antiretroviral naive persons randomized to protease inhibitor-, nonnucleoside reverse transcriptase inhibitor-, or protease inhibitor plus nonnucleoside reverse transcriptase inhibitor-based strategy. *J Acquir Immune Defic Syndr.* 2007; 44:506–517. [PubMed: 17325603]
3. Wohl D, Scherzer R, Heymsfield S, et al. The associations of regional adipose tissue with lipid and lipoprotein levels in HIV-infected men. *J Acquir Immune Defic Syndr.* 2008; 48:44–52. [PubMed: 18360291]
4. Grunfeld C, Rimland D, Gibert CL, et al. Association of upper trunk and visceral adipose tissue volume with insulin resistance in control and HIV-infected subjects in the FRAM study. *J Acquir Immune Defic Syndr.* 2007; 46:283–290. [PubMed: 18167644]
5. Masia M, Padilla S, Garcia N, et al. Endothelial function is impaired in HIV-infected patients with lipodystrophy. *Antivir Ther.* 2010; 15:101–110. [PubMed: 20167996]
6. Kosmiski LA, Bacchetti P, Kotler DP, et al. Relationship of fat distribution with adipokines in human immunodeficiency virus infection. *J Clin Endocrinol Metab.* 2008; 93:216–224. [PubMed: 17940113]
7. Guaraldi G, Stentarelli C, Zona S, et al. Lipodystrophy and anti-retroviral therapy as predictors of sub-clinical atherosclerosis in human immunodeficiency virus infected subjects. *Atherosclerosis.* 2010; 208:222–227. [PubMed: 19595354]
8. Guaraldi, G.; Zona, S.; Orlando, G., et al. 17th Conference on Retroviruses and Opportunistic Infections. San Francisco, CA: 2010. Visceral fat but not general adiposity is a predictor of cardiovascular disease in HIV-infected males.
9. Dolan SE, Hadigan C, Killilea KM, et al. Increased cardiovascular disease risk indices in HIV-infected women. *J Acquir Immune Defic Syndr.* 2005; 39:44–54. [PubMed: 15851913]
10. Triant, VA.; Meigs, JB.; Grinspoon, S. Association of C-Reactive Protein and HIV Infection with Acute Myocardial Infarction; 10th International Workshop on Adverse Drug Reactions and Lipodystrophy; November 6–8 2008; London, UK.
11. Hadigan C, Meigs JB, Rabe J, et al. Increased PAI-1 and tPA antigen levels are reduced with metformin therapy in HIV-infected patients with fat redistribution and insulin resistance. *J Clin Endocrinol Metab.* 2001; 86:939–943. [PubMed: 11158071]
12. Smith A, Patterson C, Yarnell J, Rumley A, Ben-Shlomo Y, Lowe G. Which hemostatic markers add to the predictive value of conventional risk factors for coronary heart disease and ischemic stroke? The Caerphilly Study. *Circulation.* 2005; 112:3080–3087. [PubMed: 16286603]
13. Woodward M, Rumley A, Welsh P, MacMahon S, Lowe G. A comparison of the associations between seven hemostatic or inflammatory variables and coronary heart disease. *J Thromb Haemost.* 2007; 5:1795–1800. [PubMed: 17723116]
14. Lowe GD, Danesh J, Lewington S, et al. Tissue plasminogen activator antigen and coronary heart disease. Prospective study and meta-analysis. *Eur Heart J.* 2004; 25:252–259. [PubMed: 14972427]
15. Falutz J, Allas S, Blot K, et al. Metabolic effects of a growth hormone-releasing factor in patients with HIV. *N Engl J Med.* 2007; 357:2359–2370. [PubMed: 18057338]

16. Beauregard C, Utz AL, Schaub AE, et al. Growth hormone decreases visceral fat and improves cardiovascular risk markers in women with hypopituitarism: a randomized, placebo-controlled study. *J Clin Endocrinol Metab.* 2008; 93:2063–2071. [PubMed: 18381581]
17. Johansson JO, Landin K, Johannsson G, Tengborn L, Bengtsson BA. Long-term treatment with growth hormone decreases plasminogen activator inhibitor-1 and tissue plasminogen activator in growth hormone-deficient adults. *Thromb Haemost.* 1996; 76:422–428. [PubMed: 8883281]
18. Falutz J, Allas S, Mamputu JC, et al. Long-term safety and effects of tesamorelin, a growth hormone-releasing factor analogue, in HIV patients with abdominal fat accumulation. *AIDS.* 2008; 22:1719–1728. [PubMed: 18690162]
19. Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Despres JP. A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. *American Journal of Clinical Nutrition.* 1996; 64:685–693. [PubMed: 8901786]
20. Borkan GA, Gerzof SG, Robbins AH, Hults DE, Silbert CK, Silbert JE. Assessment of abdominal fat content by computed tomography. *Am J Clin Nutr.* 1982; 36:172–177. [PubMed: 7091027]
21. Hunter GR, Snyder SW, Kekes-Szabo T, Nicholson C, Berland L. Intra-abdominal adipose tissue values associated with risk of possessing elevated blood lipids and blood pressure. *Obes Res.* 1994; 2:563–568. [PubMed: 16355516]
22. Williams MJ, Hunter GR, Kekes-Szabo T, et al. Intra-abdominal adipose tissue cut-points related to elevated cardiovascular risk in women. *Int J Obes Relat Metab Disord.* 1996; 20:613–617. [PubMed: 8817354]
23. Onat A, Avci GS, Barlan MM, Uyarel H, Uzunlar B, Sansoy V. Measures of abdominal obesity assessed for visceral adiposity and relation to coronary risk. *Int J Obes Relat Metab Disord.* 2004; 28:1018–1025. [PubMed: 15197408]
24. Ryan AS, Berman DM, Nicklas BJ, et al. Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. *Diabetes Care.* 2003; 26:2383–2388. [PubMed: 12882866]
25. He G, Andersen O, Haugaard SB, et al. Plasminogen activator inhibitor type 1 (PAI-1) in plasma and adipose tissue in HIV-associated lipodystrophy syndrome. Implications of adipokines. *Eur J Clin Invest.* 2005; 35:583–590. [PubMed: 16128865]
26. Janand-Delenne B, Chagnaud C, Raccach D, Alessi MC, Juhan-Vague I, Vague P. Visceral fat as a main determinant of plasminogen activator inhibitor 1 level in women. *Int J Obes Relat Metab Disord.* 1998; 22:312–317. [PubMed: 9578235]
27. Triant, V.; Lee, H.; Hadigan, C.; Grinspoon, S. Myocardial infarction rates among HIV-infected patients in a U.S. health care system; Presented at XVI International AIDS Society; 2006.
28. Gorog DA. Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. *J Am Coll Cardiol.* 55:2701–2709. [PubMed: 20538163]
29. Pradhan AD, LaCroix AZ, Langer RD, et al. Tissue plasminogen activator antigen and D-dimer as markers for atherothrombotic risk among healthy postmenopausal women. *Circulation.* 2004; 110:292–300. [PubMed: 15238458]
30. Gram J, Bladbjerg EM, Moller L, Sjol A, Jespersen J. Tissue-type plasminogen activator and C-reactive protein in acute coronary heart disease. A nested case-control study. *J Intern Med.* 2000; 247:205–212. [PubMed: 10692083]
31. Sam S, Haffner S, Davidson MH, et al. Relation of abdominal fat depots to systemic markers of inflammation in type 2 diabetes. *Diabetes Care.* 2009; 32:932–937. [PubMed: 19228869]
32. He G, Andersen O, Haugaard SB, et al. Plasminogen activator inhibitor type 1 (PAI-1) in plasma and adipose tissue in HIV-associated lipodystrophy syndrome. Implications of adipokines. *European Journal of Clinical Investigation.* 2005; 35:583–590. [PubMed: 16128865]
33. Koppel K, Bratt G, Schulman S, Bylund H, Sandstrom E. Hypofibrinolytic state in HIV-1-infected patients treated with protease inhibitor-containing highly active antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes: JAIDS.* 2002; 29:441–449.
34. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA.* 2004; 291:1730–1737. [PubMed: 15082700]

35. Laughlin GA, Barrett-Connor E, May S, Langenberg C. Association of adiponectin with coronary heart disease and mortality: the Rancho Bernardo study. *Am J Epidemiol.* 2007; 165:164–174. [PubMed: 17101706]
36. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol.* 2003; 23:85–89. [PubMed: 12524229]
37. Shibata R, Sato K, Pimentel DR, et al. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med.* 2005; 11:1096–1103. [PubMed: 16155579]
38. Kumada M, Kihara S, Ouchi N, et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation.* 2004; 109:2046–2049. [PubMed: 15096450]
39. Addy CL, Gavrilu A, Tsiodras S, Brodovicz K, Karchmer AW, Mantzoros CS. Hypoadiponectinemia is associated with insulin resistance, hypertriglyceridemia, and fat redistribution in human immunodeficiency virus-infected patients treated with highly active antiretroviral therapy. *J Clin Endocrinol Metab.* 2003; 88:627–636. [PubMed: 12574192]
40. Tong Q, Sankale JL, Hadigan CM, et al. Regulation of adiponectin in human immunodeficiency virus-infected patients: relationship to body composition and metabolic indices. *J Clin Endocrinol Metab.* 2003; 88:1559–1564. [PubMed: 12679439]
41. Perry A, Wang X, Goldberg R, Ross R, Jackson L. The relationship between cardiometabolic and hemostatic variables: influence of race. *Metabolism.* 2008; 57:200–206. [PubMed: 18191049]
42. Lutsey PL, Cushman M, Steffen LM, et al. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. *J Thromb Haemost.* 2006; 4:2629–2635. [PubMed: 17002663]
43. Phillips SA, Ciaraldi TP, Kong AP, et al. Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes.* 2003; 52:667–674. [PubMed: 12606507]
44. Stanley TL, Chen CY, Branch KL, Makimura H, Grinspoon SK. Effects of a Growth Hormone-Releasing Hormone Analog on Endogenous GH Pulsatility and Insulin Sensitivity in Healthy Men. *J Clin Endocrinol Metab.* 2011; 96:150, 158. [PubMed: 20943777]

Table 1**Baseline Demographics and Clinical Characteristics of the Patients**

Variables	Tesamorelin (N=273)	Placebo (N=137)
Age (years)	47.3 ± 7.3	48.3 ± 7.5
Sex (%Male / %Female)	86.8 / 13.2	83.9 / 16.1
Race (%)		
White	76.6	72.3
Black	13.6	16.1
Other	9.9	11.7
Weight (kg)	89.6 ± 14.1	90.0 ± 13.7
Body Mass Index (kg/m ²)	29.2 ± 4.2	29.0 ± 4.2
VAT (cm ²)	178 (77)	171 (77)
Waist Circumference (cm)	104 ± 9	105 ± 9
Waist-to-Hip Ratio	1.05 ± 0.06	1.05 ± 0.07
Viral Load (%)		
Undetectable	68.4	70.8
50–400 copies/mL	22.4	20.4
>400 copies/mL	9.2	8.8
CD4 cell count (cells/mm ³)	616 ± 299	585 ± 284
IGF-1 (ng/mL)	161 ± 59	168 ± 75
PAI-1 (ng/mL)	34.9 ± 19.8	35.0 ± 18.4
tPA (ng/mL)	9.7 ± 4.1	9.7 ± 3.7
CRP (mg/L)	4.6 ± 9.4	4.4 ± 6.6
Adiponectin (µg/mL)	5.3 ± 3.7	5.4 ± 3.2
Triglycerides (mg/dL)	252 ± 188	234 ± 145
Total Cholesterol (mg/dL)	197 ± 44	195 ± 38
HDL Cholesterol (mg/dL)	47 ± 15	48 ± 15
Fasting Glucose (mg/dL)	98 ± 13	97 ± 13
Fasting Insulin (µIU/mL)	19 ± 26	18 ± 11
2-h Glucose on OGTT (mg/dL)	115 ± 42	112 ± 42

Data are means ± SD, unless otherwise indicated. $P > 0.05$ for all comparisons.

Table 2

Correlation between inflammatory markers and IGF-1 and VAT at baseline

	IGF-1		VAT	
	Correlation Coefficient ^I	P-value	Correlation Coefficient ^I	P-value
PAI-1	−0.10	0.04	0.36	<0.001
tPA	−0.10	0.04	0.29	<0.001
CRP	−0.10	0.09	0.18	<0.001
Adiponectin	0.01	0.85	−0.22	<0.001

^I Spearman's rank correlation (ρ).

Table 3

Changes from Baseline to Week 26 in Inflammatory Markers

Variables	Baseline		At 26 Weeks		Absolute Difference ²	Relative Difference ³	P-Value ⁴
	Tesamorelin (N=273)	Placebo (N=137)	Tesamorelin (N=273)	Placebo (N=137)			
	Change from Baseline (Percent)						
PAI-1 (ng/mL)	34.9 ± 19.8	35.0 ± 18.4	-2.5 ± 16.9 (3.5) ^I	-1.0 ± 17.3 (8.5)	-1.5	-5.0	0.34
tPA (ng/mL)	9.7 ± 4.1	9.7 ± 3.7	-2.2 ± 2.5 (-21.6) ^I	-1.6 ± 2.9 (-14.2) ^I	-0.6	-7.4	0.03
CRP (mg/L) *	4.6 ± 9.4	4.4 ± 6.6	-0.4 ± 11.9 (23.9)	0.4 ± 7.9 (75.0)	-0.8	-51.1	0.54
Adiponectin (µg/mL) *	5.3 ± 3.7	5.4 ± 3.2	0.5 ± 2.7 (12.4) ^I	-0.1 ± 1.3 (2.4)	0.6	10	0.03

Data are reported as mean ± SD, unless otherwise indicated.

* Data previously published in [15].

^I P<0.05 for the change from baseline to Week 26 within the tesamorelin group and the placebo group.

² The values are for the difference between the changes from baseline in the tesamorelin group and the placebo group.

³ The values are for the difference between the percent change from baseline in the tesamorelin group and the placebo group.

⁴ P-values are for the comparison between the changes from baseline in the tesamorelin group and the placebo group.

Table 4

Correlation between changes in inflammatory markers and changes in VAT and IGF-I from baseline to Week 26 in the Tesamorelin Group

	Δ IGF-I		Δ %VAT	
	Correlation Coefficient ^I	P-value	Correlation Coefficient ^I	P-value
Δ PAI-1	-0.05	0.47	0.16	0.02
Δ tPA	0.05	0.47	0.16	0.02
Δ CRP	-0.11	0.11	0.11	0.12
Δ Adiponectin	0.09	0.20	-0.27	<0.001
				0.03
				0.02
				0.22
				<0.001

^I Spearman's rank correlation (ρ).