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***In Vitro* and *In Vivo* Effects of IGF-I on Adiposity in HIV-associated Metabolic Disease: A Pilot Study**

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Abstract

Background and Aims—We tested the effects of recombinant insulin-like growth factor-I (IGF-I) in an adipocyte model of HIV lipodystrophy and in an open label study on body composition and metabolism in patients with HIV lipodystrophy.

Methods—The effects of IGF-I on ritonavir-induced adipocyte cell death were studied *in vitro*. We assessed lipid accumulation, IGF signaling, apoptosis, and gene expression. We conducted a 24-week open label trial of recombinant IGF-I in ten adults with HIV associated lipodystrophy. Laboratory assessments included glucose, insulin, lipids, and IGF-I. At weeks 0 and 24, body composition studies were performed including skinfold measurement, dual-energy x-ray absorptiometry, and computed tomography of the abdomen and thigh.

Results—*In vitro*, ritonavir increased delipidation and apoptosis of adipocytes, whereas co-treatment with IGF-I attenuated the effect. In the clinical study, subcutaneous adipose tissue did not increase in patients after treatment with IGF-I; however, there was a decrease in the proportion of abdominal fat ($39.8 \pm 7\%$ vs. $34.6 \pm 7\%$, $p = 0.007$). IGF-I levels increased with treatment ($143 \pm 28 \mu\text{g/L}$ at week 0 vs. $453 \pm 212 \mu\text{g/L}$ at week 24, $p = 0.002$), whereas IGFBP-3 levels declined ($3.554 \pm 1.146 \text{ mg/L}$ vs. $3.235 \pm 1.151 \text{ mg/L}$, $p = 0.02$). Insulin at week 12 decreased significantly ($90.1 \pm 39.8 \text{ pmol/L}$ vs. $33.2 \pm 19.6 \text{ pmol/L}$, $p = 0.002$). There was a nonsignificant decrease in visceral adipose tissue ($155.2 \pm 68 \text{ cm}^2$ at week 0 vs. $140.6 \pm 70 \text{ cm}^2$ at week 24, $p = 0.08$).

Conclusions—Use of recombinant IGF-I may lower fasting insulin and abdominal fat in patients with lipodystrophy associated with HIV infection. Further evaluation of this agent for treatment of HIV-associated lipodystrophy may be warranted.

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Keywords

Lipodystrophy; Lipoatrophy; IGF-I; HIV; Mecasermin

Introduction

Metabolic complications occur frequently in HIV patients on antiretroviral therapy and can include insulin resistance, dyslipidemia, and abnormal fat redistribution (1). Although often referred to as the lipodystrophy syndrome, patients may have one, two or all of these features. Lipoatrophy, commonly affecting the face, limbs, and gluteal region, can be stigmatizing (2), affect adherence to antiretroviral therapy (3), and can worsen metabolism. Lipoatrophy exacerbates dyslipidemia and insulin resistance through changes in adipocytokines, inflammation, and lipid trafficking (1). HIV antiretroviral drugs from the nucleoside and non-nucleoside reverse transcriptase inhibitor and protease inhibitor classes are associated with metabolic complications in HIV patients (1–4). Mitochondrial toxicity and apoptosis in fat cells resulting from exposure to thymidine analog and dideoxy-nucleotide reverse transcriptase inhibitors (NRTIs) and to protease inhibitors may be pathogenic steps leading to regional loss of adipose tissue (5–8). Fat accumulation can occur concomitantly with peripheral lipoatrophy. Central fat accumulation is a feature that HIV lipodystrophy shares with the metabolic syndrome and may also worsen lipid and glucose homeostasis.

HIV patients are at risk for other endocrine abnormalities beyond those of the lipodystrophy syndrome. Impairments in the growth hormone (GH)–insulin-like growth factor-I (IGF-I) axis have been described in adult and adolescent patients with HIV infection (9–12) [ENREF 9](#). In addition, GH signaling is impaired in the setting of HIV infection (13). Growth hormone and IGF-I have been tested as treatments for HIV-associated wasting, poor growth, and to boost the immune system in HIV infection (14). Growth hormone and growth hormone-releasing hormone analogues have also been studied as treatments for HIV lipodystrophy. The results of these latter studies in lipodystrophy have generally shown a reduction in visceral fat with a neutral or negative effect on subcutaneous adiposity and glucose homeostasis (15–18).

Given that IGF-I can have anti-apoptotic properties (19) and may be impaired in HIV patients, we hypothesized that augmentation of IGF-I signaling could protect adipocytes from the toxicity of HIV antiretrovirals. Previously, we found that HIV protease inhibitors differentially induce adipocyte apoptosis *in vitro* (8). Here we present results from *in vitro* experiments examining whether IGF-I modified the survival and function of adipocytes exposed to the HIV protease inhibitor ritonavir. In addition, we present the results of a pilot study of recombinant IGF-I for adults with HIV infection and lipoatrophy.

Materials and Methods

Cell Culture

Experiments were conducted using the SGBS cell line described previously (20). The SGBS cells were a generous gift from Dr. Martin Wabitsch (University of Ulm, Germany). Cells were expanded in growth medium and differentiated into mature lipid-laden adipocytes as described previously (8). Briefly, preadipocytes are grown in DMEM/F12 media enriched with pantothenic acid, biotin, and thyroid hormone. When cells reach confluence, they are induced to differentiate by the addition of a cocktail containing troglitazone (4 μ M), dexamethasone (25 nM), cortisol (100 nM), insulin (20 nM), and isobutylmethylxanthine (500 μ M). Differentiation medium was continued for 7 days and then cells were returned to a serum-free medium supplemented with insulin and cortisol until they reached maturity, indicated by the appearance of numerous lipid droplets within each cell. Prior to experiments involving treatment with IGF-I (Sigma, St. Louis, MO), cells were pre-incubated overnight in low glucose, serum-free medium without any added hormones or growth factors.

Ritonavir was obtained from the NIH AIDS Reagent Program. Immunoblotting was performed on whole cell lysates separated by gel electrophoresis and transferred to polyvinyl fluoride membrane according to a protocol described previously (8). HDAC2 was used as a loading control as this protein was found not to change in our experimental conditions (unpublished data). Apoptosis in conditioned cells was quantitated by a commercial enzyme-linked immunosorbent assay (ELISA) for nucleosome enrichment (Roche) and by DNA fragment staining using the TdT-mediated dUTP-fluorescein nick end labeling (TUNEL) method (Roche) followed by image capture under fluorescent microscopy. IGF-I concentrations in conditioned media was determined by ELISA (R&D Systems, Minneapolis, MN). Oil Red O in methanol was used to stain cellular lipid. Cells were washed in PBS and fixed in 29% formaldehyde prior to staining for 1 h.

Gene Expression Studies

Cells were washed with PBS and then RNA was extracted using a commercial kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was generated and then gene expression was assessed by real-time quantitative polymerase chain reaction (RTqPCR) on an automated system using commercially available primer and probe assays for the genes of interest (ABI Prism 7000). Hypoxanthine phosphoribosyltransferase (HPRT) was used as the house keeping gene based on our previous studies, which found them to be stably expressed in this cell line under varying conditions (unpublished data). Gene amplification was calculated using the 2^{-C_t} method (21).

Clinical Study

Ten adult subjects with HIV infection and lipoatrophy were enrolled in an open label trial of recombinant IGF-I. To be eligible, subjects had to be ages 18–65 years, have HIV infection that was well controlled (<75 copies of HIV-1 RNA by bDNA, and have clinical evidence of lipoatrophy. Exclusion criteria included diabetes mellitus or use of a hypoglycemic agent, interferon therapy, malignancy or other serious systemic illness.

Recombinant IGF-I (mecasermin) was given at a dose of 40 µg/kg per dose by subcutaneous injection, twice daily for 24 weeks. Patients were instructed to take the medication with a meal in order to reduce the risk of hypoglycemia. The medication was generously provided by Tercica (Ipsen, South San Francisco, CA).

A fasting blood sample was obtained at weeks 0, 12, and 24. At week 0 and week 24, physical assessments were made including measurement of height, weight, skin fold thickness, and waist and hip circumference. A whole body dual-energy x-ray absorptiometry (DEXA) study was performed to assess body composition (Hologic, Bedford, MA). Single-slice computed tomography images were obtained using a standard non-contrast protocol through the abdomen at the level of the L4–L5 interspace and through the mid-thigh. A subcutaneous gluteal fat biopsy was also collected. The cross-sectional area of the adipose was determined from these CT images using public domain image analysis software (ImageJ) by an investigator (SV) blinded to subject ID and whether the image was before or after treatment. All subjects completed a body self-assessment survey before and after completing therapy. The survey assesses the severity of body fat changes on a 7-point scale. The survey has been extensively studied in this population and shown to correlate with objective body composition measurements (22,23). These surveys were generously provided by Dr. Carl Grunfeld (University of California, San Francisco). Blood samples were immediately analyzed in the clinical laboratory of the Hospital of the University of Pennsylvania (Clinical Laboratory Improvement Amendments certificate on file). IGF-I was analyzed by liquid chromatography/mass spectrometry. Inter- and intra-assay coefficients of variation were 7–8 and 6–9%, respectively, and the sensitivity to 10 µg/L. We used a range of 55–292 µg/L as the normal range of IGF-I for a 50-year-old subject (24).

Informed consent was obtained from all study participants. All human subject research procedures were approved by the Institutional Review Board of the University of Pennsylvania.

Analysis

Comparisons of continuous variables in two groups and more than two groups were performed using Students *t*-test and analysis of variance, respectively. The cell death ELISA measuring nucleosome enrichment was expressed after normalization to baseline conditions, which was set as an enrichment of 1. The primary outcome of the clinical study was adiposity in units of mass by DEXA and units of area as measured by single slice CT imaging. The secondary outcomes were biochemical including lipids, glucose, and insulin values. The differences between baseline and each follow-up interval were analyzed for significance using a two-tailed, paired *t*-test with a *p* value of 0.05. One subject withdrew from the study before 12 weeks and did not provide follow-up samples; this subject's baseline characteristics are included in the baseline univariate analysis; however, subjects' data are excluded from subsequent analyses.

Results

In vitro Experiments

As a model system for studying the effects of protease inhibitors on adipose tissue, we treated cultured human adipocytes with ritonavir. Previously, we found that ritonavir induces delipidation and cell death in mature adipocytes (8). We hypothesized that recombinant human IGF-I (rhIGF-I) added to the cell culture medium would enhance survival of adipocytes exposed to ritonavir.

Adipocytes were treated with ritonavir in the absence or presence of 100 nM rhIGF-I, a physiologically relevant concentration. Ritonavir treatment caused loss of lipid, indicated by a decrease in the intensity of oil-red-O staining (Figure 1a). Ritonavir treatment also induced apoptosis in a dose-related manner as indicated by an ELISA for nucleosome enrichment (Figure 1b). Co-treatment of ritonavir-exposed cells with recombinant IGF-I resulted in enhanced lipid content and a decrease in apoptosis (Figure 1a and 1b). Wortmannin, a PI3K inhibitor, abrogated the protective effect of IGF-I against ritonavir-induced apoptosis (Figure 1c) indicating PI3K dependence.

Adipocytes pre-treated with ritonavir showed a diminished IGF-I stimulated Akt phosphorylation response after 20 h (Figure 2a). Because human adipocytes are known to express and secrete IGF-I (25), we also examined the effect of ritonavir on these aspects of adipocyte function. Ritonavir reduced IGF-I mRNA expression in adipocytes (Figure 2b) and suppressed IGF-I protein secretion at 48 h (Figure 2c).

To examine the effects of insulin and IGF-I on adipocyte survival, ritonavir-exposed cultured adipocytes were co-incubated with varying concentrations of insulin and IGF-I. Cell death was examined by TUNEL staining. Insulin and IGF-I reduced cellular apoptosis at physiologically relevant ranges. Insulin and IGF-I in combination had the strongest effect on attenuating cell apoptosis (Figure 3). In summary, ritonavir caused a loss of lipid and induced adipocyte apoptosis, impaired IGF-I signaling and also decreased adipocyte IGF-I gene expression and protein secretion. Treatment of ritonavir-exposed fat cells with exogenous IGF-I preserved lipid content and enhanced fat cell survival.

Clinical Study Results

Baseline demographic and clinical characteristics of the subjects are shown in the Table 1. Nine of ten subjects were male and the mean age was 52 years (median 50.5 years). At study entry, all subjects were on a nucleoside reverse transcriptase inhibitor, and 80% were on protease inhibitors. All patients on protease inhibitors were taking ritonavir as a boosting agent for a second protease inhibitor. The antiretroviral combinations taken by each subject at study entry are shown in Table 1. All patients maintained an undetectable HIV RNA level throughout the study. Their baseline CD4 counts are shown in Table 1. The CD4 count was statistically unchanged during the study, going from 581 ± 240 cells/mm³ at week 0 to 526 ± 202 cells/mm³ at week 24 ($p = 0.4$).

Mean IGF-I levels were normal at baseline (24) and increased significantly during the treatment period (Table 2). There was a concomitant decrease in IGFBP3, probably resulting

from a downregulation of growth hormone secretion by the action of IGF-I. Fasting insulin levels decreased from 90.1 pmol/L at study entry to 33.2 pmol/L at 12 weeks, a 63% decrease ($p = 0.002$). Fasting insulin levels seemed to “rebound” at 24 weeks in some individuals, although there remained a trend towards a persistent reduction ($p = 0.07$). The fasting glucose value, measured at baseline and week 24, did not change significantly during the study. Total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol did not change significantly during treatment. Triglyceride levels were lower at week 12 and week 24; however, the differences from baseline did not reach statistical significance (Table 2).

Body weight, body mass index, waist circumference, hip circumference and waist to hip ratio did not change significantly in subjects (Table 3). Skin fold measurements did not significantly change (data not shown). According to DEXA scan, the amount of limb fat and total fat did not change significantly. Fat free mass was not changed. Average central fat mass decreased 5.5% but did not reach statistical significance ($p = 0.1$) (Table 4). By self-assessment, 6/9 subjects who reported moderate or severe buccal fat wasting at baseline reported no change after 24 weeks of treatment. Three of nine subjects reported improvement in buccal wasting. Five of eight subjects reporting gluteal lipoatrophy at baseline reported no change at study end, whereas three reported subjective improvement. Four subjects reported a subjective decrease in waist size at the end of the study, two reported no change, two reported an increase, and one was unsure if there had been a change in waist size.

Cross-sectional CT imaging of the abdomen indicated a decrease in abdominal fat. Total abdominal fat decreased from 290–259 cm² although the change did not reach statistical significance ($p = 0.08$). Abdominal fat as a percentage of total area decreased significantly from 39.8–34.6% ($p = 0.007$). Intra-abdominal adipose area decreased from 155.–140.6 cm² ($p = 0.08$). Neither abdominal nor thigh subcutaneous fat area changed significantly after treatment (Table 5).

Gene Expression

We examined the gene expression in adipose tissue biopsies to assess whether there was evidence for changes in apoptosis or modulators of insulin sensitivity. Specifically, we performed RTqPCR for the genes encoding Bax and Bcl-2, which are proteins that regulate apoptosis and for the gene encoding adiponectin, a fat-secreted hormone that enhances glucose homeostasis. No significant changes in expression were found for any of these genes (data not shown).

Safety and Follow-up

No subjects had symptoms of hypoglycemia. One subject with a pre-existing history of headaches reported headaches during the study. This subject’s dose was lowered by 50% and continued for the remainder of the study. One subject withdrew due to social stressors unrelated to the study. This subject did not have assessments at weeks 12 or 24 and therefore was not included in the paired analyses.

Discussion

Fat redistribution is associated with metabolic abnormalities and cosmetic distress for HIV patients. In particular, facial fat loss can be stigmatizing for its sufferers and decrease adherence to antiretroviral therapy (26). Lipoatrophy is also associated with an abnormal profile of adipocytokine secretion, which may exacerbate insulin resistance and dyslipidemia (27,28).

Evidence for increased apoptosis has been found in the adipose tissue of patients with HIV lipodystrophy (29). This could be a result of mitochondrial toxicity; however, a causal relationship has not been clearly established (30). Among the antiretroviral drug classes, protease inhibitors and nucleoside reverse transcriptase inhibitors have been implicated as potential offenders; however, the effects vary by specific agents within a class. The *in vitro* data presented here suggest that IGF-I may be protective in adipocytes exposed to ritonavir through a PI3K-dependent mechanism. Given that HIV patients may have diminished GH/IGF-I activity, we studied whether supplementing IGF-I could improve body composition and metabolism in patients with HIV-associated lipodystrophy syndrome.

Administration of rhIGF-I resulted in significant elevations in IGF-I; on average supraphysiological levels (24) of this hormone were achieved. Concomitantly, there was a small but statistically significant decrease in IGFBP-3. This most likely resulted from negative feedback of rising IGF-I levels on pituitary GH secretion. A similar finding was described when rhIGF-I was given to obese type 2 diabetics (31). In contrast, rhIGF-I caused no change in IGFBP-3 when given to patients with type 1 diabetes mellitus (32). The reasons for variation in IGFBP-3 response in these conditions are not completely clear but may be related to portal insulin levels that modulate hepatic response to GH (33). Long-term elevations of IGF-I could be concerning in HIV patients due to a theoretical increased risk of cancer. This is an important consideration in clinical trials using agents of the GH-IGFI axis in HIV patients. In addition, suppression of GH secretion by administration of recombinant IGF-I could result in metabolic changes due to the loss of direct GH effects on its target tissues. The consequences of low GH could include decreases in fat oxidation, tolerance to fasting, and tissue anabolism (34). After treatment with IGF-I, fasting insulin levels decreased suggesting enhanced insulin sensitivity. Interestingly, this occurred without any change in body weight or fat free mass. The insulin-lowering effect of IGF-I may be mediated by suppressed GH secretion, a hormone that induces insulin resistance through a post-receptor mechanism (35,36). Alternatively, a direct effect of IGF-I on the liver to decrease hepatic glucose output may also be a factor (37,38). In our patients, another factor that may have reduced fasting insulin levels is the decrease in the relative amount of abdominal fat. In contrast to the change in fasting insulin, there was no clear change in any lipid parameter following IGF-I treatment. Although insulin decreased, fasting triglycerides did not decline as has been reported when rhIGF-I was given to patients with type 1 diabetes mellitus (39). A lack of change in fasting glucose and lipids was contrary to expectations. It is possible that some of the metabolic changes due to rhIGF-I treatment may accrue only after greater body composition changes occur, which could take a longer duration of treatment. Another explanation is that changes occurred only in patients with intrinsic insufficiency in the GH/IGF-I axis. Our sample population was too low to study this

hypothesis in a rigorous manner. However, in the future, testing patients for GH/IGF-I axis dysfunction may be a way to identify those most likely to benefit from this type of hormonal therapy.

There are several limitations to this study. First, this study was a pilot single-arm treatment protocol. Therefore, it is possible that the observed changes were due to other factors unrelated to receiving rhIGF-I. Although no subjects reported changes in physical activity or diet, and there were no changes in body weight or lean mass, we cannot rule out other exposures as a cause for the decrease in abdominal fat. The number of subjects treated was small; therefore, a type II error may account for the lack of statistically significant changes in triglycerides, fat distribution, and body composition. Facial and gluteal fat were not measured quantitatively and thus could have changed in a way not captured by our assessments.

Surprisingly, assessments of abdominal fat suggested a decrease in visceral fat following rhIGF-I treatment. Intra-abdominal fat as a proportion of total abdominal area decreased significantly and there was a trend towards a decrease in visceral adipose area and central fat mass. This was an unexpected observation because IGF-I signals through a pathway that overlaps that of insulin, which is an adipogenic hormone (40). Furthermore, owing to negative feedback on the pituitary, endogenous growth hormone would be predicted to be low although was not directly measured. Previous studies of growth hormone and growth hormone-releasing hormone analogues in HIV patients with lipodystrophy indicate a predominantly lipolytic effect of GH (41,42). The finding that IGF-I administration was associated with loss of abdominal adiposity is also unexpected given the results of our *in vitro* experiments, which suggested an adipose-sparing effect. This surprising discrepancy remains unexplained but may result from differing effects of IGF-I on visceral vs. subcutaneous adipocytes or because of other changes in the *in vivo* hormonal milieu such as a decrease in cortisol or 11 β -hydroxysteroid dehydrogenase 1 that were not replicated *in vitro*.

Limb fat and subcutaneous adipose did not change appreciably in our patients following IGF-I treatment. We found no evidence for a change in the expression of genes related to apoptosis in the adipose tissue of patients. Because adipose tissue turns over slowly (43), the number of apoptotic events in adipose may be so low that changes in their expression escaped our detection methods. However, no subjects felt there was a worsening of facial or gluteal lipoatrophy, and several felt there was some restoration of fat in these areas although these were subjective ratings.

In summary, these data suggest that IGF-I may reduce fasting insulin levels and decrease visceral adiposity in patients with HIV lipodystrophy. Additional study of this agent in this patient population should be conducted in randomized, placebo-controlled fashion.

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Figure 1.

(A) Ritonavir treatment results in loss of lipid content in cultured human adipocytes. Co-treatment with recombinant IGF-I preserves lipid content in adipocytes exposed to ritonavir. Mature human adipocytes were treated for 1 week with ritonavir at the concentrations shown, and also with or without rhIGF-I (100 nM). (B) Apoptosis is increased in adipocytes with higher concentrations of ritonavir (circle). Co-treatment with recombinant IGF-I significantly lowers apoptosis (triangle) (* $p = 0.01$). Adipocytes were treated overnight with ritonavir with or without rhIGF-I. Apoptosis was measured by a nucleosome ELISA kit (Roche). (C) Wortmannin blockade of PI3K function abrogates the protective effect of IGF-I. Cells were treated overnight with ritonavir alone (circle), ritonavir plus 100 nM IGF-I (triangle), or ritonavir and IGF-I and wortmannin (square). (*triangle vs. square, $p = 0.02$). Nucleosome enrichment was measured by ELISA. (Inset) Wortmannin, a PI3K inhibitor, blocks IGF-I-stimulated Akt phosphorylation.

Figure 2.

(A) Ritonavir reduces IGF-I stimulated phosphor-Akt signal in adipocytes compared to control conditions in which adipocytes were treated only with IGF-I without preceding ritonavir exposure. Adipocytes were incubated overnight in vehicle or ritonavir (50 μ M) and then stimulated with recombinant IGF-I for the time indicated time. Western blotting was performed using phosphor-Akt antibody. Ritonavir treated cells have lower intensity phospho-Akt signal at 20 h suggesting earlier attenuation of signal. (B) Ritonavir causes a dose-related decrease in IGF-I gene expression in adipocytes compared to control (*10 μ M vs. control: $p = 0.001$; **50 μ M vs. control: $p = 0.01$). (C) Ritonavir (10 μ M = square; 50 μ M = triangle) induced a transient decrease in IGF-I secretion (*10 μ M vs. control [circle]: $p = 0.06$; and 50 μ M vs. control: $p = 0.004$).

Figure 3.

Insulin and IGF-I reduce adipocyte apoptosis as ascertained by TUNEL staining. Adipocytes were incubated for 48 h in ritonavir plus varying concentrations of insulin and/or IGF-I. The cells were then examined after TUNEL staining. TUNEL positive and total cells were counted, and the results normalized using vehicle control as a reference of 1. Mean TUNEL positivity was compared between groups using ANOVA ($p = 0.001$). Data shown are the results of three separate experiments with each condition tested in at least triplicate.

Patient characteristics (*n* = 10)

Table 1

Subject #	Age (years)	Gender ^a	Ethnicity ^b	Antiretroviral regimen	Baseline CD4 count
1	50	M	C	darunavir raltegravir ritonavir tenofovir/ emtricitabine	487
2	61	M	C	abacavir nevirapine tenofovir	500
3	60	M	C	lopinavir/ritonavir tenofovir/ emtricitabine atazanavir	381
4	51	M	C	ritonavir zidovudine/ lamivudine	456
5	46	M	C	darunavir tenofovir ritonavir	852
6	48	M	C	efavirenz lopinavir/ritonavir tenofovir	212
7	55	M	C	fosamprenavir ritonavir tenofovir/ emtricitabine	949
8	51	M	C	efavirenz/tenofovir/ emtricitabine	712
9	47	F	AA	abacavir atazanavir ritonavir	830
10	47	M	C	lopinavir/ritonavir tenofovir/ emtricitabine	432
Mean (SD)	51.9 (5.5)	-	-	-	581 (240)

^a M, male; F, female.

^b C, Caucasian; AA, African-American.

Table 2

Hormonal and biochemical parameters during recombinant IGF-I therapy

	Week 0	Week 12	Week 24
IGF-I, µg/L	143 (28)	417 (181)*	453 (212)**
IGFBP3, mg/L	3.554 (1.161)	3.123 (1.509)**	3.235 (1.151)**
Insulin, pmol/L	90.1 (39.8)	33.2 (19.6)*	62.8 (49.9)***
Glucose, mmol/L	4.6 (0.89)	-	4.6 (0.67)
Triglycerides, mmol/L	2.12 (0.96)	1.80 (0.95)	1.83 (1.37)
Total cholesterol, mmol/L	4.75 (0.57)	4.72 (0.62)	4.54 (0.94)
LDL-C, mmol/L	2.88 (0.47)	2.98 (0.49)	2.79 (0.7)
HDL-C, mmol/L	0.90 (0.21)	0.93 (0.27)	0.91 (0.24)

Mean (SD) shown.

*
 $p < 0.003$.**
 $p = 0.02$.***
 $p = 0.07$.

Table 3

Anthropometric changes during mecamermin therapy

Parameter	Week 0	Week 24	% change	<i>p</i>
Weight, kg	84.5 (12)	83.8 (12)	−1.6%	0.12
BMI	26.5 (4.1)	26.3 (4.3)	−0.9%	0.4
Waist, cm	95.3 (8.4)	95.6 (7.5)	+0.5%	0.8
Hip, cm	97.7 (8.3)	96.3 (7.9)	−1.3%	0.18
WHR	0.98 (0.05)	0.99 (0.05)	+1.8%	0.13

Mean (SD) shown.

Table 4

Body composition by DEXA

Parameter	Week 0	Week 24	% Change	<i>p</i>
Limb fat, g	6,286 (5141)	6,180 (4972)	−1.4	0.6
Central fat, g	9,085 (2721)	8,595 (2905)	−5.5	0.1
Total fat, g	16,436 (7525)	15,835 (7575)	−3.8	0.3
Total fat free mass, g	65,350 (6215)	65,591 (7341)	+0.2	0.8

Table 5

Abdominal and thigh adiposity measured by CT

	Week 0	Week 24	<i>p</i>
Total abdominal fat, cm ²	290.0 (97)	258.7 (98)	0.08
Total abdominal fat %	39.8 (7)	34.6 (7)	0.007
VAT, cm ²	155.2 (68)	140.6 (70)	0.08
SAT, cm ²	134.9 (82)	118.1 (76)	0.38
Thigh subcutaneous fat, cm ²	69.9 (89)	66.4 (85)	0.57

CT, computed tomography; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.