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Preliminary Report

Leptin replacement improves postprandial glycemia and insulin sensitivity in human immunodeficiency virus–infected lipoatrophic men treated with pioglitazone: a pilot study

Faidon Magkos[a,*], *Aoife Brennan*[a], *Laura Sweeney*[a], *Eun Seok Kang*[a], *John Doweiko*[b], *Adolf W. Karchmer*[b], *Christos S. Mantzoros*[a]

[a] *Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA*
[b] *Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*

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ABSTRACT

Highly active antiretroviral therapy (HAART)–induced lipoatrophy is characterized by hypoleptinemia and insulin resistance. Evidence suggests that pioglitazone and recombinant methionyl human leptin (metreleptin) administration has beneficial effects in human immunodeficiency virus (HIV)–infected lipoatrophic patients. This proof-of-concept study aimed at evaluating whether the combination of metreleptin and pioglitazone has favorable effects, above and beyond pioglitazone alone, on both metabolic outcomes and peripheral lipoatrophy in HIV-infected patients on HAART. Nine HIV-positive men with at least 6 months of HAART exposure, clinical evidence of lipoatrophy, and low leptin concentrations (≤4 ng/mL) were placed on pioglitazone treatment (30 mg/d per os) and were randomized to receive either metreleptin (0.04 mg/kg subcutaneously once daily; n = 5) or placebo (n = 4) for 3 months in a double-blinded fashion. Compared with placebo, metreleptin reduced fasting serum insulin concentration, increased adiponectin concentration, reduced the homeostasis model assessment index of insulin resistance, and attenuated postprandial glycemia in response to a mixed meal (all $P \leq .02$), but did not affect trunk and peripheral fat mass. HIV control was not affected, and no major adverse effects were observed. Metreleptin administration in HIV-positive, leptin-deficient patients with lipoatrophy treated with pioglitazone improves postprandial glycemia and insulin sensitivity. Results from this pilot study should be confirmed in larger clinical trials.

1. Introduction

Highly active antiretroviral therapy (HAART) significantly reduces disease-associated mortality and morbidity in patients with human immunodeficiency virus (HIV) infection; however, its use has been linked with multiple metabolic abnormalities including insulin resistance, dyslipidemia, and body fat redistribution (lipodystrophy), thereby increasing the risk of cardiovascular disease [1,2]. Lipoatrophy, characterized by generalized fat depletion, is present in 15% to 35% of HIV patients on HAART [3] and is associated with hyperinsulinemia, dyslipidemia, and hypoleptinemia [4].

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* *Corresponding author*. Harvard Medical School, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA. Tel.: +1 617 667 7118; fax: +1 617 667 8634.
E-mail address: fmagkos@bidmc.harvard.edu (F. Magkos).

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Treatment with pioglitazone, a peroxisome proliferator-activated receptor–γ agonist with insulin-sensitizing properties, tends to increase total body fat and limb fat mass in HIV-infected patients; but its effects on insulin sensitivity are inconsistent [5-7]. We [8] and others [9] have demonstrated that administration of recombinant methionyl human leptin (metreleptin) improves insulin resistance but also reduces total body fat in lipoatrophic HIV patients. The reduction in total body fat was not likely accompanied by worsening of peripheral lipoatrophy because it was mainly attributed to reduced central fat deposition [8,9]. In the present proof-of-concept study, we hypothesized that the addition of metreleptin to pioglitazone treatment would result in a therapeutic benefit above and beyond that observed on pioglitazone alone.

2. Methods

2.1. *Subjects*

Nine adult men (age, 50 [range, 43-57] years; body mass index, 26 [range, 23-29] kg/m^2) with HIV-1 infection and at least 6 months of cumulative HAART exposure, serum leptin concentrations less than or equal to 4 ng/mL (mean, 2.6 ng/mL), and clinical evidence of lipoatrophy (developed after the initiation of HAART) were recruited sequentially from the infectious disease and primary care clinics at the Beth Israel Deaconess Medical Center (BIDMC) and the Boston community via advertisement. Exclusion criteria were history of fasting hyperinsulinemia, hyperglycemia, impaired glucose tolerance, type 2 diabetes mellitus, and dyslipidemia before the initiation of HAART; abnormal hepatic and renal functions; active infection other than HIV; overt hypo- and hyperthyroidism; hypogonadism; hypercortisolism; treatment with steroids or growth hormone; as well as alcoholism and drug abuse. The study was approved by the BIDMC Institutional Review Board, and subjects provided written informed consent.

2.2. *Experimental protocol and study outcomes*

All subjects followed the American Heart Association Step 2 diet for a 4-week lead-in phase. Thereafter, pioglitazone treatment was started (30 mg/d per os); and subjects were randomized in a double-blind fashion to receive either metreleptin (n = 5; 0.04 mg/kg per day, self-injected subcutaneously once daily between 7:00 PM and 11:00 PM) or placebo (n = 4; same volume and timing as leptin) for 3 months; metreleptin and placebo were supplied by Amylin Pharmaceuticals (San Diego, CA). Study outcomes were assessed before and after treatment. On each occasion, subjects arrived in the laboratory in the morning, following an overnight fast. After collection of a baseline blood sample, a standard low-fat mixed meal test (Boost; 240 kcal, 17% from protein, 68% from carbohydrate, and 15% from fat) was administered to evaluate postprandial glucose and insulin metabolism. Body fat mass and fat distribution were determined by using dual-energy x-ray absorptiometry (QDR-4500; Hologic, Bedford, MA). The next morning, after measurement of resting blood pressure and heart rate, 7 subjects (3 on placebo and 4 on metreleptin) underwent a short (50 minutes), insulin-modified (0.03 U/kg), intravenous glucose (300 mg/kg) tolerance test [10]. Subjects were instructed to maintain their HAART regimen, physical activity, and dietary habits during the course of the study.

2.3. *Sample analysis and calculations*

Commercially available assays were used for measuring serum concentrations of leptin, adiponectin (both from Linco Research, St Charles, MO), and insulin (Diagnostic Products, Los Angeles, CA). Serum glucose, interleukin-6, glycosylated hemoglobin, total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, and total triglyceride concentrations; liver function indices; complete blood cell counts; and HIV markers (HIV viral load, CD4 and CD8 lymphocyte counts) were assayed by using standard methods at the BIDMC clinical laboratory. Trunk fat mass was estimated from the whole-body dual-energy x-ray absorptiometry scan [8]. Areas under the concentration-vs-time curves for glucose and insulin in response to the mixed meal were calculated by using the trapezoidal rule. The homeostasis model assessment (HOMA) index was calculated to evaluate insulin resistance [11].

2.4. *Statistical analysis*

Statistical analyses were performed with Predictive Analytics Software version 18.0 (SPSS, Chicago, IL). Differences between groups at baseline were assessed with the Mann-Whitney *U* test. To evaluate the effect of metreleptin treatment compared with placebo, changes from baseline were compared between groups by using nonparametric multivariate analysis of variance on rank scores, adjusting for the number of outcome variables. Both on-treatment (n = 6; 3 on placebo and 3 on metreleptin) and intention-to-treat (n = 9; 1 on placebo and 2 on metreleptin whose missing values were replaced by carrying forward the last observation) analyses were performed. Descriptive data are presented as means ± standard error or means with 95% confidence interval from the intention-to-treat analysis. A 2-tailed P value < .05 was considered significant.

3. Results

At baseline, there were no significant differences between metreleptin- and placebo-treated subjects in body mass index (P = .56), body weight (P = .29), age (P = .33), and all clinical and metabolic parameters ($P \geq .11$). All patients maintained their HAART regimen, physical activity, and dietary habits stable throughout the study (self-report). Treatment with metreleptin increased serum leptin concentrations (3.7 ± 0.1 to 16.5 ± 6.7 ng/mL) compared with placebo (1.9 ± 0.7 to 3.4 ± 1.2 ng/mL) (P = .02).

One subject treated with metreleptin and one treated with placebo withdrew from the study because of mild injection site reactions; another subject on metreleptin dropped out for personal reasons unrelated to the study procedures. Overall, no major adverse effects were observed.

Metreleptin significantly reduced fasting serum insulin concentration and the HOMA index, but did not affect intravenous glucose disappearance, fasting serum glucose concentration, or

glycosylated hemoglobin (Table 1). Compared with placebo, metreleptin significantly reduced postprandial glycemia without affecting the insulinemic response to the meal (Fig. 1).

Treatment with metreleptin did not affect body mass index, body fat mass and distribution, resting blood pressure and heart rate, fasting serum lipid profile, and fasting interleukin-6 concentration, but increased serum adiponectin concentration compared with placebo (Table 1).

Changes in hematology parameters (white and red blood cells, hematocrit, hemoglobin, platelets, total lymphocytes) as well as HIV markers (viral load, CD4 and CD8 lymphocyte counts) did not differ significantly between metreleptin and placebo (all $P \geq .33$; data not shown). However, alkaline phosphatase was significantly reduced by metreleptin; and a similar though not significant change was observed for liver transaminases (Table 1).

4. Discussion

Previous studies have shown that leptin replacement in humans with HIV-related lipoatrophy improves hyperinsulinemia and insulin resistance, reduces total body and trunk fat, mildly improves lipid profile, and does not affect peripheral fat [8,9], whereas pioglitazone increases total body and peripheral fat with no consistent effects on insulin sensitivity and blood lipid profile [5-7]. In this pilot study, we found that metreleptin reduces fasting insulin concentration and insulin resistance (ie, HOMA) and attenuates postprandial glycemia in HIV-positive subjects treated with pioglitazone.

It was recently demonstrated that metreleptin administration increases insulin sensitivity in the liver (ie, suppression of glucose production) but not skeletal muscle (ie, stimulation of glucose uptake) [9]. This is in agreement with our finding of reduced fasting insulin and HOMA index because HOMA mainly reflects hepatic, not muscle, insulin resistance [12]. Furthermore, hepatic glucose production is almost completely suppressed during the intravenous glucose tolerance test; therefore, intravenous glucose disappearance primarily reflects skeletal muscle insulin sensitivity [13], whereas, following ingestion of a mixed meal, hepatic glucose production is only marginally suppressed [14]. Collectively, these observations together with our findings suggest that metreleptin improves postprandial glycemia and insulin sensitivity, possibly by acting at the level of the liver. The increase in adiponectin concentration after metreleptin treatment may also contribute to this

Table 1 – Study outcomes before and after 3 months of placebo or metreleptin administration in HIV-infected lipoatrophic men treated with pioglitazone

	Placebo (n = 4)		Metreleptin (n = 5)		Treatment effect		P[a]	P[b]
	Before	After	Before	After	Placebo	Metreleptin		
Body composition								
Body mass index (kg/m^2)	26 ± 1	26 ± 1	27 ± 1	27 ± 1	0.0 (–0.2, 0.3)	0.1 (–0.3, 0.5)	.85	1.00
Body fat (%)	17 ± 1	17 ± 1	20 ± 1	20 ± 1	0.2 (–0.9, 1.2)	–0.3 (–1.0, 0.4)	.55	.46
Total body fat (kg)	13.0 ± 0.5	13.1 ± 0.8	17.0 ± 1.5	16.7 ± 1.7	0.16 (–0.85, 1.18)	–0.28 (–0.87, 0.31)	.55	.46
Peripheral fat (kg)	3.8 ± 0.2	3.8 ± 0.2	5.2 ± 0.6	5.1 ± 0.7	0.02 (–0.07, 0.11)	–0.03 (–0.17, 0.10)	.55	.81
Trunk fat (kg)	8.1 ± 0.4	8.2 ± 0.6	10.6 ± 1.3	10.4 ± 1.3	0.15 (–0.79, 1.09)	–0.22 (–0.65, 0.22)	.55	.46
Insulin sensitivity								
Fasting glucose (mg/dL)	119 ± 24	122 ± 23	116 ± 19	110 ± 15	2.8 (–3.3, 8.8)	–5.6 (–27.6, 16.4)	.32	.49
Fasting insulin (mU/L)	3.9 ± 0.7	5.7 ± 2.6	8.5 ± 1.9	6.1 ± 2.3	1.8 (–4.5, 8.1)	–2.4 (–6.6, 1.9)	.02	.08
Glycosylated hemoglobin (%)	5.9 ± 0.5	5.9 ± 0.5	5.9 ± 0.2	5.7 ± 0.2	0.0 (–0.2, 0.3)	–0.1 (–0.4, 0.1)	.14	.17
HOMA insulin resistance	1.2 ± 0.4	1.8 ± 0.8	2.3 ± 0.6	1.6 ± 0.5	0.6 (–1.3, 2.4)	–0.7 (–1.7, 0.3)	.02	.08
Glucose disappearance slope (%)	–2.9 ± 0.9	–2.4 ± 0.7	–2.9 ± 0.6	–3.2 ± 0.6	–0.2 (–1.5, 1.1)	0.2 (–0.5, 0.8)	.64	.56
Cardiovascular system								
Systolic blood pressure (mm Hg)	121 ± 7	124 ± 4	128 ± 11	127 ± 12	3 (–10, 15)	–1 (–11, 8)	.57	.49
Diastolic blood pressure (mm Hg)	75 ± 7	75 ± 5	82 ± 6	81 ± 6	0.3 (–8, 9)	–1 (–8, 6)	1.00	.73
Heart rate (beats/min)	79 ± 7	70 ± 7	79 ± 6	78 ± 5	–9 (–45, 27)	–1 (–6, 3)	1.00	.91
Lipid profile								
Total cholesterol (mg/dL)	188 ± 18	183 ± 17	176 ± 11	190 ± 13	–5 (–47, 38)	14 (–18, 46)	.33	.65
Triglyceride (mg/dL)	239 ± 102	184 ± 101	179 ± 68	175 ± 71	–56 (–211, 99)	–4 (–18, 10)	.33	.49
HDL cholesterol (mg/dL)	38 ± 2	44 ± 5	36 ± 1	38 ± 3	6 (–7, 20)	1 (–5, 7)	.33	.22
LDL cholesterol (mg/dL)	107 ± 18	108 ± 7	104 ± 11	117 ± 8	0.3 (–45, 46)	13.6 (–16, 43)	.33	.65
Adipocytokines								
Adiponectin (μg/mL)	1.6 ± 0.3	1.9 ± 0.5	2.5 ± 0.4	4.0 ± 0.9	0.3 (–1.2, 1.7)	1.6 (–0.2, 3.4)	.02	.08
Interleukin-6 (pg/mL)	3.1 ± 0.8	5.2 ± 2.4	2.2 ± 0.5	2.0 ± 0.6	2.1 (–5.2, 9.4)	–0.3 (–0.6, 0.1)	.57	.35
Serum liver enzymes								
Alanine transaminase (U/L)	33 ± 4	45 ± 17	32 ± 4	26 ± 2	12 (–50, 74)	–5 (–13, 2)	.85	.82
Aspartate transaminase (U/L)	35 ± 3	27 ± 3	27 ± 3	23 ± 2	–8 (–25, 9)	–4 (–9, 1)	.85	.65
Alkaline phosphatase (U/L)	84 ± 8	83 ± 9	85 ± 14	78 ± 14	–0.8 (–7.5, 6.0)	–6.4 (–14.7, 0.9)	.01	.07

Values before and after treatment are means ± standard error; difference scores are means with 95% confidence intervals. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

[a] P value from on-treatment analysis.

[b] P value from intention-to-treat analysis.

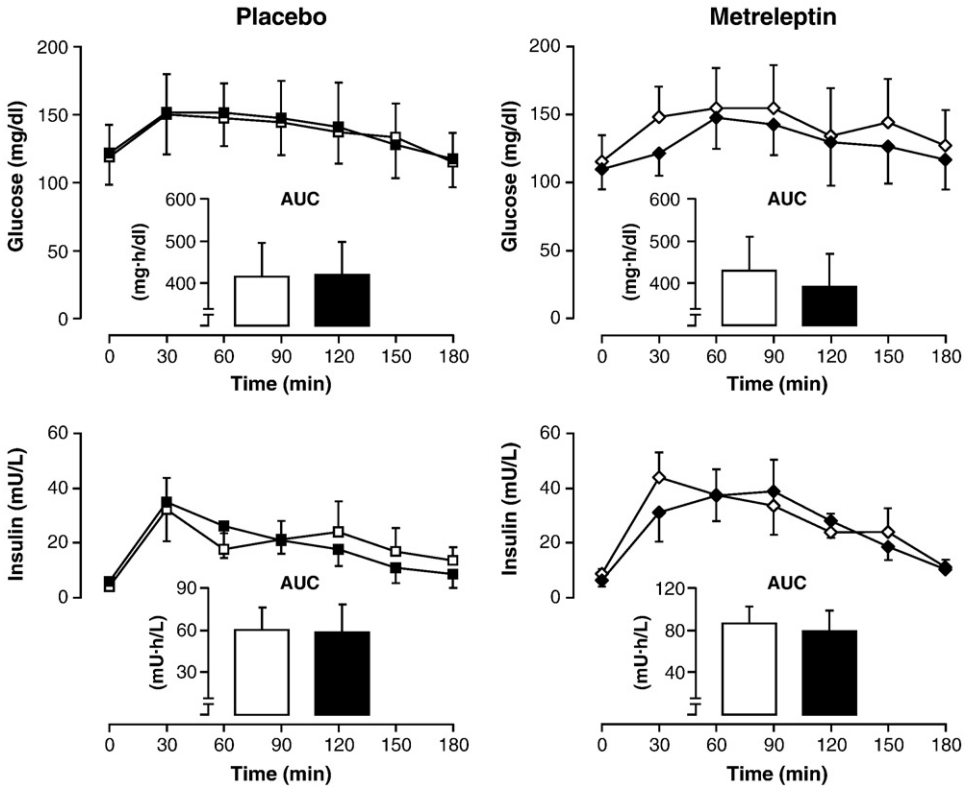

Fig. 1 – Glucose (top) and insulin (bottom) concentrations and areas under the curve in response to a mixed meal before (white symbols) and after (black symbols) 3 months of placebo or metreleptin administration in HIV-infected lipoatrophic men treated with pioglitazone. Compared with placebo, metreleptin significantly attenuated postprandial glycemia ($P = .02$ and $P = .01$ for the on-treatment and intention-to-treat analyses, respectively), but did not affect postprandial insulinemia ($P = .57$ and $P = .82$, respectively).

effect because adiponectin has been previously shown to directly correlate with the insulin-mediated suppression of hepatic glucose production in HIV-infected patients [15]. The reduction in serum alkaline phosphatase and perhaps in liver transaminases indicates that metreleptin resulted in an overall improvement in liver function, although values for all these enzymes were within reference range in both groups, whether before or after treatment.

Limited available studies indicate that pioglitazone favorably affects peripheral lipoatrophy in HIV-infected patients by increasing total body and limb fat mass [5-7], whereas metreleptin reduces central fat deposition [8,9]. We hypothesized that treatment with metreleptin plus pioglitazone would preserve these beneficial effects. Although metreleptin decreased total body and trunk fat in all subjects on treatment, the effect was small (~0.3 kg) and did not approach significance; and we found no indication of an improvement in peripheral lipoatrophy. This could possibly be due to the short duration of our study, the low metreleptin dose, and/or the small number of subjects. Earlier reports provide evidence suggesting that the effects of metreleptin may be both time [8] and dose [9] dependent. It is also likely that the duration of our experiment (3 months) was too short for pioglitazone-induced changes in body composition to manifest; previous studies reporting beneficial effects administered the drug for longer periods of time (6-12 months) [5-7]. In fact, in one of these studies where interim measurements were obtained at 3 months, only trends but no statistically significant effects of pioglitazone on body fat distribution were observed [6]. Longer-term administration (≥6 months) may thus be necessary to detect the putative beneficial effects of pioglitazone. It is also possible that the opposite effects of metreleptin [8,9] and pioglitazone [5-7] on body fat cancel out when both drugs are administered. Therefore, the combination of metreleptin with pioglitazone may retain the insulin-sensitizing and hypoglycemic effects of metreleptin while at the same time prevent the decrease in fat mass induced by metreleptin alone, and needs to be further studied by larger trials.

In conclusion, results from this pilot study indicate that leptin replacement attenuates fasting insulinemia and insulin resistance and improves postprandial glycemia in lipoatrophic HIV-positive men treated with pioglitazone. These preliminary findings have important clinical implications for the treatment of HIV-related lipoatrophy and metabolic syndrome, and need to be replicated in larger and longer-term studies.

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REFERENCES

[1] Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. N Engl J Med 2005;352:48-62.
[2] Leow MK, Addy CL, Mantzoros CS. Human immunodeficiency virus/highly active antiretroviral therapy–associated metabolic syndrome: clinical presentation, pathophysiology, and therapeutic strategies. J Clin Endocrinol Metab 2003;88:1961-76.
[3] Jacobson DL, Knox T, Spiegelman D, et al. Prevalence of, evolution of, and risk factors for fat atrophy and fat deposition in a cohort of HIV-infected men and women. Clin Infect Dis 2005;40:1837-45.
[4] Nagy GS, Tsiodras S, Martin LD, et al. Human immunodeficiency virus type 1–related lipoatrophy and lipohypertrophy are associated with serum concentrations of leptin. Clin Infect Dis 2003;36:795-802.
[5] Calmy A, Hirschel B, Hans D, et al. Glitazones in lipodystrophy syndrome induced by highly active antiretroviral therapy. AIDS 2003;17:770-2.
[6] Gavrila A, Hsu W, Tsiodras S, et al. Improvement in highly active antiretroviral therapy-induced metabolic syndrome by treatment with pioglitazone but not with fenofibrate: a 2 × 2 factorial, randomized, double-blinded, placebo-controlled trial. Clin Infect Dis 2005;40:745-9.
[7] Slama L, Lanoy E, Valantin MA, et al. Effect of pioglitazone on HIV-1–related lipodystrophy: a randomized double-blind placebo-controlled trial (ANRS 113). Antivir Ther 2008;13:67-76.
[8] Lee JH, Chan JL, Sourlas E, et al. Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy. J Clin Endocrinol Metab 2006;91:2605-11.
[9] Mulligan K, Khatami H, Schwarz JM, et al. The effects of recombinant human leptin on visceral fat, dyslipidemia, and insulin resistance in patients with human immunodeficiency virus-associated lipoatrophy and hypoleptinemia. J Clin Endocrinol Metab 2009;94:1137-44.
[10] Tura A, Sbrignadello S, Succurro E, et al. An empirical index of insulin sensitivity from short IVGTT: validation against the minimal model and glucose clamp indices in patients with different clinical characteristics. Diabetologia 2010;53:144-52.
[11] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[12] Tripathy D, Almgren P, Tuomi T, et al. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. Diabetes Care 2004;27:2204-10.
[13] Vallerand AL, Frim J, Kavanagh MF. Plasma glucose and insulin responses to oral and intravenous glucose in cold-exposed humans. J Appl Physiol 1988;65:2395-9.
[14] Capaldo B, Gastaldelli A, Antoniello S, et al. Splanchnic and leg substrate exchange after ingestion of a natural mixed meal in humans. Diabetes 1999;48:958-66.
[15] Reeds DN, Yarasheski KE, Fontana L, et al. Alterations in liver, muscle, and adipose tissue insulin sensitivity in men with HIV infection and dyslipidemia. Am J Physiol Endocrinol Metab 2006;290:S47-S53.