Inhibition of lipolysis improves insulin sensitivity in protease inhibitor–treated HIV-infected men with fat redistribution1–3

Colleen Hadigan, Jessica Rabe, Gary Meininger, Negar Aliabadi, Jeffrey Breu, and Steven Grinspoon

ABSTRACT
Background: Fatty acid concentrations are increased in patients with HIV and fat redistribution and may contribute to insulin resistance in this population.

Objective: We determined the effects of acute inhibition of lipolysis on insulin sensitivity in HIV-infected patients with fat redistribution who were receiving a protease inhibitor.

Design: Seven HIV-infected men [age: 45 ± 2 y; body mass index (in kg/m²): 28.8 ± 1.9] with a fasting insulin concentration ≥104 pmol/L (15 μIU/mL), combined visceral adiposity and peripheral lipodystrophy, and receiving a protease inhibitor were studied. Tolbutamide-modified frequently sampled intravenous-glucose-tolerance tests (FSIGTTs) were performed after randomized double-blind administration of acipimox (500 mg at −90 and 0 min), a potent inhibitor of lipolysis, and placebo. The subjects completed 2 FSIGTTs separated by 3–7 d.

Results: At baseline, fasting insulin and fatty acid concentrations were 27.6 ± 5.0 μIU/mL and 0.83 ± 0.08 mmol/L (normal range: 0.1–0.6 mmol/L), respectively. Fatty acid concentrations were significantly reduced after acipimox compared with placebo (fatty acid area under the curve: acipimox = 73 ± 8 compared with placebo = 122 ± 12 mmol · 270 min/L, P = 0.002). Acipimox treatment resulted in a significant increase in the insulin sensitivity index (acipimox = 1.63 ± 0.5 compared with placebo = 0.88 ± 0.3 × 10⁻³ · min⁻¹ · μIU/mL, P = 0.015).

Conclusions: Acute inhibition of lipolysis and reduction in fatty acid concentrations are associated with improved insulin sensitivity in patients with HIV lipodystrophy and hyperinsulinemia. Further studies are needed to determine whether long-term antilipolytic strategies to reduce fatty acid concentrations may be useful in treating the metabolic disturbances associated with HIV lipodystrophy. Am J Clin Nutr 2003;77:490–4.

KEY WORDS Lipodystrophy, insulin resistance, fatty acids, acipimox, HIV, men

INTRODUCTION

A syndrome of fat redistribution, or lipodystrophy, associated with dyslipidemia and insulin resistance is increasingly recognized among HIV-infected patients and is estimated to affect more than one-half of all patients receiving combination antiretroviral therapy (1). HIV-infected patients with fat redistribution, characterized by increased visceral adiposity and subcutaneous fat atrophy, are at increased risk of impaired glucose tolerance and diabetes (2). The mechanisms of insulin resistance in HIV-infected patients treated with highly active antiretroviral therapy remain unknown. In vitro data suggest a direct effect of protease inhibitors to decrease glucose transport (3). However, clinical data also indicate a strong relation between the degree of fat redistribution and hyperinsulinemia in affected patients (2, 4, 5).

A potential mechanism by which increased visceral adiposity and subcutaneous fat loss may result in insulin resistance is through increased concentrations of circulating fatty acids, which in turn may contribute to hepatic insulin resistance and decreased insulin signaling through insulin receptor substrate 1 (6). For example, among non-HIV-infected patients with congenital lipodystrophy and in animal models of lipodystrophy (A-ZIP/F-1 mice), fatty acid concentrations are elevated and are thought to contribute to insulin resistance (7, 8). Increased fatty acid concentrations were shown in HIV-infected patients with fat redistribution and insulin resistance (9, 10). Furthermore, we recently showed that fatty acid concentrations are a strong predictor of insulin response to standard oral glucose challenge in HIV-infected patients with lipodystrophy, independent of the effects of age, body mass index (BMI), sex, and body composition (10). To test the hypothesis that increased circulating fatty acids contribute to insulin resistance in patients with HIV infection and lipodystrophy, we determined the effect of acute inhibition of lipolysis and fatty acid reduction on insulin sensitivity in HIV-infected men with fat redistribution and hyperinsulinemia. Our data, showing significant improvement in insulin sensitivity after an acute lipolytic blockade, suggest the importance of long-term studies of therapies to decrease lipolysis and improve insulin resistance in this population.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from the Massachusetts General Hospital and community-based infectious disease practices. Eligibility was based on the following inclusion criteria: documented HIV

1 From the Neuroendocrine Unit (CH, JR, GM, NA, and SG) and the Program in Nutritional Metabolism (CH, GM, NA, and SG), Massachusetts General Hospital, Boston; the Clinical Research Center, Massachusetts Institute of Technology, Cambridge (JR); and Harvard Medical School, Boston (CH, GM, and SG).

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TABLE 1
Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th>Value</th>
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<tbody>
<tr>
<td>Age (y)</td>
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<tr>
<td>Duration of HIV infection (y)</td>
</tr>
<tr>
<td>Duration of antiretroviral therapy (y)</td>
</tr>
<tr>
<td>Duration protease inhibitor therapy (y)</td>
</tr>
<tr>
<td>CD4+ count (cells/mm³)</td>
</tr>
<tr>
<td>HIV viral RNA (copies/mL)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
</tr>
<tr>
<td>Extremity fat (%)</td>
</tr>
<tr>
<td>Trunk fat (%)</td>
</tr>
<tr>
<td>Visceral adipose tissue (mm²)</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue (mm²)</td>
</tr>
<tr>
<td>Visceral adipose tissue:total adipose tissue</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
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<tr>
<td>LDL (mmol/L)</td>
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<tr>
<td>HDL (mmol/L)</td>
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<tr>
<td>Triacylglycerols (mmol/L)</td>
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</tbody>
</table>

† ± SEM; n = 7.

### Protocol

After eligibility was established, the subjects returned for 2 test days separated by 3–7 d. Each day the subjects reported to the Clinical Research Center after a 12-h overnight fast and completed a frequently sampled intravenous-glucose-tolerance test (FSIGTT, 11). One day the subjects received acipimox (500 mg orally; Olbec-tam Pharmacia, Milan, Italy) at −90 min and again at 0 min during the FSIGTT; on the other day the subjects received an identical placebo at the same time points. Two doses were administered 90 min apart to provide maximal lipolytic blockade for the duration of the FSIGTT. The dosing interval was based on the pharmacologic half-life of acipimox. The order of acipimox and placebo was randomized, and both the investigators and subjects were blinded to the order of drug assignment. Randomization was performed by the Massachusetts General Hospital pharmacy.

The tolbutamide-modified FSIGTT as developed by Bergman et al (11) was used. The FSIGTT provides a precise measure of insulin sensitivity and was shown to correlate well with euglycemic insulin clamp studies in populations with insulin resistance (12, 13). A bolus of 25% glucose solution (0.5 g/kg, with a maximal dose of 35 g) was administered over 1–3 min at the start of the study. Intravenous tolbutamide was given 20 min after glucose administration at the following doses: subjects with a BMI (in kg/m²) < 30 received 300 mg, and subjects with a BMI ≥ 30 received 500 mg. Venous blood samples were obtained at −15, −10, −5, −1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, and 180 min for the determination of plasma glucose and insulin concentrations. Insulin sensitivity and acute insulin response to glucose were calculated from these values with the nonlinear mathematical model of glucose disappearance (11). Fatty acid concentrations were determined at −90 min and every 30 min thereafter for the remainder of the testing period.

Cross-sectional abdominal computed tomography scans were performed as described by Borkan et al (14) to assess the distribution of subcutaneous and visceral adipose tissue. A lateral scout image was obtained to identify the level of the L4 pedicle, which served as the landmark for the 1-cm single-slice image. The subjects also underwent total-body dual-energy X-ray absorptiometry with the Hologic QDR-4500A scanner (Hologic Inc, Bedford, MA). The DXA scan was used to determine total-body fat and lean mass as well as regional body fat measurements, such as percentage trunk fat (trunk fat/total trunk mass) and percentage extremity fat (extremity fat/total extremity mass).

### Biochemical and immunologic assays

Concentrations of glucose, cholesterol, triacylglycerol, HDL, and LDL were determined by methods previously reported (2). Nonsug-terified fatty acid concentrations were measured by using an in vitro enzymatic colorimetric assay kit (Wako Chemicals USA, Inc, Richmond, VA). The intraassay CV for fatty acids ranged from 1.1% to 2.7%. The published normal range for fatty acids is 0.1–0.6 mmol/L. Insulin concentrations were measured in serum by radioimmunoassay (Diagnostic Product Corp, Los Angeles). The intraassay and interassay CVs ranged from 4.7% to 7.7% and from 5.5% to 9.2%, respectively. The CD4+ count was determined by flow cytometry (Becton Dickinson Immunocytochemistry Systems, San Jose, CA), and the HIV viral load was determined by ultrasensitive assay (Amplitcor HIV-1 Monitor Assay; Roche Molecular Systems, Branchburg, NJ), with limits of detection of 50–75,000 copies/mL.

### Statistical analyses

Univariate clinical characteristics are presented as means ± SEMs. The primary outcome variables were insulin sensitivity index (SI) and fatty acid area under the curve during an FSIGTT. The SI was calculated by using MINIMAL MODEL IDENTIFICATION (version 3.0, developed by Richard Bergman, 1994). The effect of acipimox on these outcome variables was determined by using paired t tests, comparing results after acipimox to placebo for each subject. Confirmatory analyses were performed with a nonparametric Wilcoxon rank-sum test. Simple linear regression was used to assess the relation between continuous variables (eg, SI and fatty acid area under the curve). All statistical analyses were performed with SAS JMP (version 3.2.2; SAS Institute, Inc, Cary, NC), and statistical significance was defined as a two-tailed α value of P < 0.05.

### RESULTS

Seven men with HIV infection, hyperinsulinemia, and significant fat redistribution completed the protocol. Clinical characteristics of the subjects are summarized in Table 1. Each subject was...
receiving a protease inhibitor and at least one nucleoside reverse transcriptase inhibitor; only one patient was receiving a nonnucleoside reverse transcriptase inhibitor. The subjects showed significant central adiposity (waist-to-hip ratio: 0.99 ± 0.01) as well as extremity fat loss, which was rated as moderate to severe in all cases. The basal fasting insulin concentration was 27.6 ± 8 mmol/L, and the baseline fatty acid concentration was increased above the normal range for the assay (0.83 ± 0.08 mmol/L; normal range: 0.1–0.6 mmol/L).

Acipimox administration significantly reduced fatty acid concentrations compared with placebo (Figure 1). The fatty acid area under the curve was significantly different after acipimox (73 ± 8 mmol · 270 min/L) than after placebo (122 ± 12 mmol · 270 min/L), \( P = 0.002 \) (paired \( t \) test). \( n = 7 \).

Acipimox was well tolerated in this study. One subject developed moderate flushing ≤ 30 min after receiving acipimox, but this resolved spontaneously ≤ 1 h. Another subject reported developing a transient rash 3 d after receiving acipimox that resolved spontaneously in 1 d and was not believed to be related to the medication.

**DISCUSSION**

Metabolic abnormalities, including insulin resistance, dyslipidemia, and fat redistribution are a significant problem for many HIV-infected patients receiving combination antiretroviral therapy. Although specific medications may affect glucose homeostasis directly (15), it is equally plausible that substantial changes in fat distribution contribute to insulin resistance in these patients. Fatty acid concentrations are increased in HIV-infected patients with fat redistribution and contribute to impaired glucose tolerance in this population (9, 10). Increased basal lipolytic rates were recently shown to be a strong predictor of insulin resistance in HIV-infected men (16). Furthermore, Johnson et al (17) showed increased basal lipolysis in cultured adipocytes from HIV-infected patients with fat redistribution.

Our data suggest that HIV-infected patients with significant visceral adiposity and peripheral subcutaneous fat loss, selected on the basis of fasting hyperinsulinemia, show increased fatty acid concentrations. To test the hypothesis that increased lipolysis and fatty acids contribute to insulin resistance among HIV-infected patients, we investigated the effects of lipolytic blockade in this population. Treatment with acipimox, a potent nicotinic acid analogue, resulted in decreased fatty acid concentrations in our study population. Acipimox is widely used as a lipid-lowering therapy for the treatment of hypercholesterolemia and hypertriglyceridemia, and the presumed mechanism of action is through reduction in release of fatty acids and decreased VLDL cholesterol.

The subjects in the present study, all of whom showed evidence of fat redistribution and who were receiving a protease inhibitor, were highly insulin resistant, with a reduced basal SI (SI = 0.88). With the use of FSIGTT, Kosmiski et al (18) previously showed a similarly low SI (SI = 1.00) in 14 protease inhibitor–treated HIV-infected patients with lipodystrophy. In the present study, acute inhibition of lipolysis resulted in an almost 2-fold increase in insulin sensitivity in HIV-infected patients with fat redistribution and hyperinsulinemia. Importantly, fatty acid concentrations after acipimox administration were inversely associated with insulin sensitivity during FSIGTT, suggesting that the significant increase in insulin sensitivity after acipimox is indeed an effect of inhibition of lipolysis. However, despite a significant increase, the SI after acipimox remained low (1.63 ± 0.05). The persistent low insulin sensitivity may be caused by several factors in this population. All the subjects were required to be receiving a protease

**FIGURE 1.** Mean (± SEM) fatty acid concentrations during the frequently sampled intravenous-glucose-tolerance test after administration of placebo (○) and acipimox (■; 500 mg/dose). The fatty acid area under the curve was significantly different after acipimox (73 ± 8 mmol · 270 min/L) than after placebo (122 ± 12 mmol · 270 min/L), \( P = 0.002 \) (paired \( t \) test). \( n = 7 \).

**FIGURE 2.** Insulin sensitivity index during the frequently sampled intravenous-glucose-tolerance test after placebo and acipimox (\( n = 7 \)). \( P \) for placebo compared with acipimox = 0.015 (paired \( t \) test).
thiazolidinediones activate peroxisome proliferator activated receptor g and stimulate adipogenesis, resulting in reduced lipolysis (7). Our data provide a rationale for the investigation of the thiazolidinediones to improve fat redistribution and insulin resistance in HIV-infected patients, in whom acute reduction in lipolysis significantly improves insulin sensitivity.

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REFERENCES


