Original Article

Inhibition of Lipolysis Stimulates Peripheral Glucose Uptake but Has No Effect on Endogenous Glucose Production in HIV Lipodystrophy

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HIV-infected patients with lipodystrophy (HIV lipodystrophy) are insulin resistant and have elevated plasma free fatty acid (FFA) concentrations. We aimed to explore the mechanisms underlying FFA-induced insulin resistance in patients with HIV lipodystrophy. Using a randomized, placebo-controlled, cross-over design, we studied the effects of an overnight acipimox-induced suppression of FFAs on glucose and FFA metabolism by using stable isotope–labeled tracer techniques during basal conditions and a two-stage euglycemic-hyperinsulinemic clamp (20 and 50 mU insulin/m^2 per min, respectively) in nine patients with nondiabetic HIV lipodystrophy. All patients received antiretroviral therapy. Biopsies from the vastus lateralis muscle were obtained during each stage of the clamp. Acipimox treatment reduced basal FFA rate of appearance by 68.9% (95% CI 52.6–79.5) and decreased plasma FFA concentration by 51.6% (42.0–58.9) (both, $P < 0.0001$). Endogenous glucose production was not influenced by acipimox. During the clamp, the increase in glucose uptake was significantly greater after acipimox treatment compared with placebo (acipimox: 26.85 μmol · kg^{-1} · min^{-1} [18.09–39.86] vs. placebo: 20.30 μmol · kg^{-1} · min^{-1} [13.67–30.13]; $P < 0.01$). Insulin increased phosphorylation of Akt Thr^{308} and glycogen synthase kinase-3β Ser^9, decreased phosphorylation of glycogen synthase (GS) site 3a + b, and increased GS activity (percent I-form) in skeletal muscle ($P < 0.01$). Acipimox decreased phosphorylation of GS (site 3a + b) ($P < 0.02$) and increased GS activity ($P < 0.01$) in muscle. The present study provides direct evidence that suppression of lipolysis in patients with HIV lipodystrophy improves insulin-stimulated peripheral glucose uptake. The increased glucose uptake may in part be explained by increased dephosphorylation of GS (site 3a + b), resulting in increased GS activity. *Diabetes* 56:2070–2077, 2007

HIV-associated lipodystrophy (HIV lipodystrophy) is characterized by various degrees of subcutaneous fat loss, visceral fat accumulation, and metabolic disturbances, including peripheral and hepatic insulin resistance (1–3) and elevated plasma free fatty acids (FFAs) (4). Increased plasma FFA concentration is due to increased lipolysis (5,6), with increased net release of FFAs from adipose tissue (5,7). In other insulin-resistant states (e.g., obesity and type 2 diabetes), increased rates of lipolysis and plasma FFA concentration are related to the degree of insulin resistance (8–10). Both acute and chronic suppression of lipolysis can improve insulin sensitivity in HIV lipodystrophy (11,12). However, the nature of improved insulin resistance (i.e., whether due to changes in hepatic or peripheral insulin sensitivity) has not been determined, and the molecular mechanism behind FFA-induced insulin resistance is still incompletely understood. FFAs have been proposed to induce skeletal muscle insulin resistance by inhibiting insulin-stimulated glucose transport/phosphorylation (13–17) and glycogen synthesis (13–15,17–20). Recently, it has been demonstrated that FFAs interfere with the proximal steps of the insulin signaling cascade by impairing the insulin-stimulated tyrosine phosphorylation of insulin receptor substrate (IRS)-1, IRS-1–associated phosphatidylinositol-3-kinase activity (13,16,21–24), and phosphorylation of Akt (Ser^{473} and Thr^{308}) (21,24), resulting in decreased GLUT4 translocation to the plasma membrane. FFAs also influence the distal part of the insulin pathway. Previous studies (13–15,17–19) consistently find that FFAs can reduce glycogen synthesis via inhibition of insulin-stimulated glycogen synthase (GS) activity, but the mechanism behind reduced GS activity is unknown (24).

In HIV lipodystrophy, both oxidative and nonoxidative glucose metabolism are impaired (25–27). A recent study (27) shows that patients with HIV lipodystrophy have a decreased insulin-stimulated GS activity in skeletal muscle. This defect in insulin action is associated with reduced dephosphorylation on sites 2 + 2a, 3a, and 3a + b, most likely via impaired signaling at the level of glycogen synthase kinase (GSK)-3α Ser^{21} and GSK-3β Ser^9 and Akt

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ARV, antiretroviral; FFA, free fatty acid; G6P, glucose-6-phosphate; GS, glycogen synthase; GSK, glycogen synthase kinase; IRS, insulin receptor substrate.

but not further upstream at the level of IRS-1–associated phosphatidylinositol-3-kinase activity (27).

The present study aimed to explore the mechanisms underlying the increase in insulin sensitivity after acute suppression of lipolysis by acipimox in patients with HIV lipodystrophy and fasting hyperinsulinemia. We hypothesized that acipimox treatment would enhance insulin sensitivity by reducing endogenous glucose production and increasing peripheral glucose uptake. Furthermore, we hypothesized that increased insulin-stimulated peripheral glucose uptake would involve improved distal insulin signaling at the level of Akt, GSK-3, and GS in muscle.

RESEARCH DESIGN AND METHODS

Twenty-one HIV-positive men were recruited from the outpatient clinic of the Department of Infectious Disease (Rigshospitalet, Copenhagen, Denmark). Subjects underwent a medical examination, and standard blood tests were performed to determine eligibility. The following were the inclusion criteria: *1*) >18 years of age; *2*) stable antiretroviral (ARV) therapy, with no changes in ARV therapy during the preceding 8 weeks; and *3*) fasting plasma insulin ≥104 pmol/l. If the fasting plasma insulin concentration was <104 ρmol/l, a standard 75-g oral glucose tolerance test was made and the subject was included if insulin levels were >521 ρmol/l after 120 min (11,28); *4*) lipoatrophy (at least one moderate sign of lipoatrophy [fat loss] in face, arms, buttocks, or legs based on a physical examination by a single investigator [B.L.]). The degree of lipoatrophy was rated as previously described (29).

The exclusion criteria were *1*) severe cardiovascular diseases; *2*) hospitalization within the last 8 weeks; *3*) fasting glucose ≥7 mmol/l; and *4*) concurrent therapy with insulin, antidiabetes agents, testosterone, growth hormone, glucocorticoids, anabolic steroids, or anticoagulation. Of 21 patients who were recruited, 11 fulfilled the inclusion criteria and 2 declined to participate. Consequently, nine patients were included in the study. The following were reasons for exclusion: plasma insulin <104 ρmol/l ($n = 6$), severe cardiovascular diseases ($n = 2$), and no lipodystrophy by score ($n = 2$). Two of six patients with plasma insulin <104 ρmol/l had lipodystrophy by the lipodystrophy score system. All patients received a combination of ARV therapies. All, except two, received protease inhibitors. Written informed consent was obtained according to the requirements from the local ethical committee.

Body composition. Fat and fat-free tissue masses of the whole body, trunk, and extremities were measured using a dual-energy X-ray absorptiometry scanner (Norland XR 36, software version 3.94; Norland, Fort Atkinson, WI) (1).

Stable isotope infusion study. The study was a randomized, double-blind, cross-over study. Each subject was studied twice (at least 2 weeks apart), once while receiving acipimox and once while receiving placebo (Fig. 1). The ARV medication was taken 3.5–4.5 h before commencing the clamp, and the ARV combination was not changed throughout the study. Acipimox was administered in doses of 250 mg at 7:00 P.M. and 12:00 A.M. the night before and at 7:00 A.M. and 9:00 A.M. on the study day (30,31). At 7:00 A.M. on the study day after an overnight fast, an intravenous catheter was inserted into an antecubital vein of one arm for infusion of stable isotope–labeled tracers, insulin, and dextrose (20%). A second intravenous catheter was inserted into a hand vein of the contralateral arm, which was wrapped in a heating blanket to obtain arterialized blood samples. After baseline blood samples were obtained at 8:00 A.M., a primed 16-μmol/kg constant infusion (0.22 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of [6,6-$^2H_2$]-glucose (Cambridge Isotopes Laboratories, Cambridge, MA) was started and maintained for 8.5 h to determine glucose kinetics. At 9:30 A.M., infusion of [2,2-$^2H_2$]-palmitate (Cambridge Isotopes Laboratories) at a constant rate of 0.04 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ was started to determine fatty acid kinetics, as previously described (3,32,33). At 10:30 A.M., 2.5 h after the start of the isotope infusion (basal period), a two-stage euglycemic-hyperinsulinemic clamp was initiated and continued for 6 h, modified after the protocol (34). During stage 1 of the clamp (from 10:30 A.M. to 12:30 P.M.), insulin (Actrapid 100 IU/ml; Novo Nordisk) was infused at a rate of 20 mU/m^2 per min (initiated with a two-step priming dose of 80 mU/m^2 per min for 5 min followed by 40 mU/m^2 per min for 5 min). During stage 2 of the clamp (from 12:30 to 4:30 P.M.), insulin was infused at a rate of 50 mU/m^2 per min (initiated with a two-step priming dose of 200 mU/m^2 per min for 5 min followed by 100 mU/m^2 per min for 5 min). Euglycemia was achieved by a variable rate of infusion of 20% glucose enriched to ~2.5% with [6,6-$^2H_2$]-glucose to maintain a blood glucose concentration at 5.5 mmol/l. The infusion of [6,6-$^2H_2$]-glucose was decreased by 50% of the initial infusion rate during stage 1 and 75% of basal during stage 2 to steadily maintain the plasma glucose enrichment by accounting for the expected decline in hepatic glucose production. The infusion of [2,2-$^2H_2$]-palmitate was decreased by 50% of the initial infusion rate during stages 1 and 2 because of the expected decline in whole-body lipolytic rate. One subject could not complete the clamp study for technical reasons. Results during the clamp therefore represent data from eight subjects only.

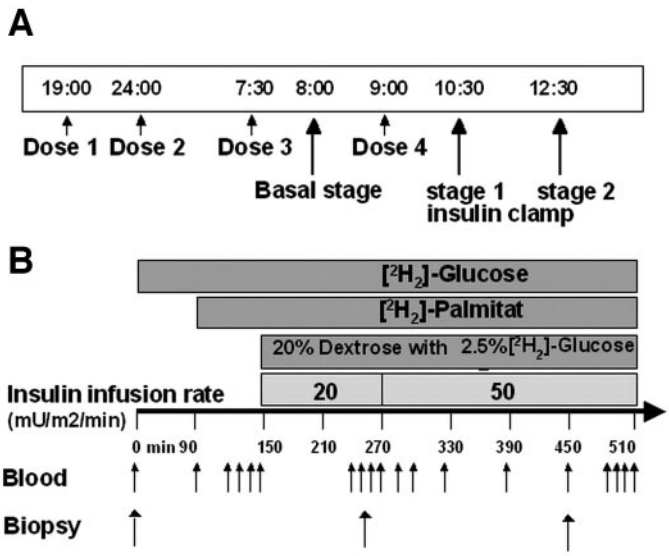

FIG. 1. Schematic representation of the study. *A*: Administration of acipimox/placebo and time schedule. Acipimox and placebo were administered overnight. *B*: The experimental study day. Whole-body glucose metabolism was measured by combined euglycemic-hyperinsulinemic clamp and stable isotope tracer method. Insulin was infused for 120 min at a rate of 20 mU/m^2 per min (stage 1) and for 240 min at a rate of 50 mU/m^2 per min (stage 2). Steady-state measurements of glucose and palmitate concentrations and kinetics were carried out during the last 30 min of the basal period and during each stage of the insulin clamp every 10 min. Arrows show time points for blood and skeletal muscle biopsy samples.

Muscle biopsy. Muscle biopsies from m. vastus lateralis were obtained with a modified Bergström needle (including suction) under local anesthesia with 2% lidocaine within the first 30 min of the basal stage and at the end of stages 1 and 2 of the clamp. Muscle tissue was immediately frozen in liquid nitrogen and stored at −80°C.

Blood samples. Arterialized blood samples were taken before beginning the isotope infusion to determine background glucose and palmitate enrichments. To determine glucose and palmitate concentrations and kinetics, arterialized blood samples were taken every 10 min during the last 30 min of the basal period and each stage of the insulin clamp (i.e., basal: 120, 130, 140, and 150 min; stage 1: 240, 250, 260, and 270 min; and stage 2: 480, 490, 500, and 510 min after the start of the tracer infusion) (3,32) (Fig. 1). Venous blood samples were taken at 0, 30, 90, 120, 150, 180, 210, 240, 270, 300, 330, 390, 450, and 510 min. All blood samples were drawn into tubes containing EDTA and centrifuged. Plasma was stored at −80°C. Plasma concentration of FFAs and glycerol were determined using an automatic analyzer (Cobas fara; Roche, Basel, Switzerland; FFA: NEFA C; Wako Chemicals, Neuss, Germany). Insulin was measured by enzyme-linked immunosorbent assay (detection limit, 3.0 pg/ml; DakoCytomation, Cambridgeshire, U.K.). All determinations were run as duplicates, and mean values were calculated. To maintain euglycemia, arterialized blood glucose was measured every 5 min in the 1st hour of each clamp stage and every 10 min throughout the rest of the clamp and was analyzed immediately (EML 105 radiometer; Radiometer Medical A/S, Copenhagen, Denmark).

Total, HDL, and LDL cholesterol (mmol/l); triglycerides (mmol/l); and glucose (mmol/l) measurements on the day of inclusion were determined using standard laboratory procedures. CD4$^+$ cell counts were calculated by flow cytometry and HIV RNA copies (lower limit of detection: 20 copies/ml) were measured by the Amplicor HIV Monitor (Roche Molecular Systems, Branchburg, NJ).

Tracer analyses

Glucose. Enrichment of glucose was measured as previously described in detail (35). In short, 20 μl plasma was vigorously shaken with 100 μl ethyl acetate to remove plasma lipids. After centrifugation (15,000 rpm at 5 min) the ethyl acetate was disposed and 20 μl 8 mol NaOH, 1 mol K_2HPO_4, and 10 μl benzoylchlorid were added, and the solution was vortexed for 5 min. After the

vortex, the solution was naturalized by adding 10 μl 1.4 H_3PO_4, and the glucose derivate, hexo-benzoyl glucose, was extracted by a short vigorous shake with 500 μl ethylacetate. The ethylacete phase was separated by centrifugation and transferred to a new eppendorf tube, and the ethylacetate was evaporated under a stream of nitrogen. The glucose derivate was dissolved in the high-performance liquid chromatography buffer. The solution was filtered before being analyzed on the liquid chromotography–mass spectrometry system (aQa; Finnegan, Manchester, U.K.).

Palmitate. Concentration and enrichment was determined by gas chromatography–mass spectrometry analysis (Automass II; Finnegan, Paris, France) as described by Patterson et al. (36).

The fatty acid concentrations were determined by using an internal standard (1,2,3,4-$^{13}C_4$-palmitic acid; Sigma Aldrich, MO), which was related to a standard curve. To determine plasma palmitate enrichment, the sample was separated on a 30-m capillary fused-silica column (Rtx-2330; Restex, Forney, TX) with an injector temperature at 50°C increased to 200°C at a rate of 20°C per min. The oven was set at the following program: initial 50°C increasing to 150°C at 10°C per min and then slowly increased to 163°C with a rate of 2°C per min. To clean the column, the temperature was increased to 260°C at a rate of 20°C per min and maintained at 260°C for 7 min. The mass spectrometer was scanned in a profile mode in a range from 287 to 311 amu.

Calculations. Tracer-to-tracee ratios for both glucose and palmitate were calculated as the ratio between the master peak (M) and the enriched peak (M + 2) after subtracting the background enrichment. The calculations are based on a steady-state assumption. For glucose the total rate of appearance (R_a) was calculated as the following:

$$R_{a(endogenous)} = \frac{F_{total}}{E_{glucose}} - GIR \quad (1)$$

and R_d as the following:

$$R_d = \frac{F_{total}}{E_{glucose}} \quad (2)$$

where R_a is rate of appearance (μmol · kg body wt^{-1} · min^{-1}). F_{total} is the total infusion rate of glucose tracer (μmol · kg^{-1} · min^{-1}). $E_{glucose}$ is the enrichment of glucose in plasma (tracer-to-tracee ratio). *GIR* is the exogenous glucose administered during the clamp. R_d is rate of disappearance (μmol · kg body wt^{-1} · min^{-1}).

For palmitate, R_a was calculated as the follwing:

$$R_a = R_d = \frac{F}{E_{palmitate}}$$

where F is the infusion rate of $^2H_2$-palmitate (μmol · kg body wt^{-1} · min^{-1}) and $E_{palmitate}$ is the tracer-to-tracee ratio of plasma palmitate. The exact amount of tracer in the infusate was measured, as described above, for plasma palmitate concentration and enrichment, and this value was used in the calculation. R_a is equal to R_d during isotopic and physiologic steady state.

SDS-PAGE and Western blotting. Muscle homogenate proteins were separated using 7.5% Bis-tris gels (Invitrogen, Taastrup, Denmark) and transferred (semidry) to polyvinylidine fluoride membranes (immobilon transfer membrane; Millipore, Glostrup, Denmark). After blocking (Tris-buffered saline with Tween with 2% skim milk), the membranes were incubated with primary antibodies (Tris-buffered saline with Tween with 2% skim milk) followed by incubation in horseradish peroxidase–conjugated secondary antibody (Tris-buffered saline with Tween with 2% skim milk) (Amersham Pharmacia Biotech, Buckinghamshire, U.K.). Following detection and quantification using a charge-coupled device image sensor and 1D software (Kodak Image Station, 2,000 mm; Kodak, Ballerup, Denmark), the protein content was finally expressed in arbitrary units relative to a human skeletal muscle standard. The antibodies used for detection of phosphorylation of AKT and GSK-3 were as follows: AKT Thr^{308} (Upstate Biotechnology, Lake Placid, MA) and GSK-3β Ser^9 (~35-kDa band using the anti–GSK-3α/β Ser^{21}/Ser^9 pan antibody; New England Biolabs, Beverly, MA). For detection of phosphorylation of glycogen synthase at site 3a + 3b (Ser^{640}) and Ser^{644}, an antibody raised in sheep, as described (37), was used.

GS activity. GS activity was measured in muscle homogenates using a Unifilter 350 microtiter plate assay (Whatman, Frisenette, Ebeltoft, Denmark). The assay ran in triplicate based on the original protocol described by Thomas et al. (38). GS activity was determined in the presence of 8 or 0.02 mmol/l glucose-6-phosphate (G6P), representing the maximal GS activity and the G6P-independent activity, respectively. The percent I-form of GS was calculated as 100 times the activity in the presence of 0.02 mmol/l G6P divided by the activity at 8.0 mmol/l G6P.

Statistics. Values of FFAs, glycerol, R_a, R_d, and insulin were naturally log transformed to achieve homoscedacity and an approximate normal distribution. GS activity (I-form) and GSK-3β were analyzed with and without log transformation. Statistical analyses of substrate concentration and kinetics were carried out using a general linear mixed model. A random subject-specific component was introduced that allowed adjusting for the interindividual variations. The effect of treatment was estimated using a binary variable (acipimox or placebo) and the effect of insulin infusion using a categorical variable (none, low [20 units/l], or high [50 units/l]) infusion level), and the model allowed for an interaction term between the two where needed. Time in minutes was entered as a continuous variable in the analysis of glucose, insulin, FFAs, and glycerol. Likelihood ratio tests were applied to assess statistical significance. The fit of the general linear model was evaluated by testing the residuals for normality and by inspection of the residual plots. Linear regression was used to quantify the proportion of the effect of increased dephosphorylation of GS site 3a + b on R_d that could be explained by acipimox by the decrease in the effect estimate when treatment entered the model. The model was adjusted for insulin infusion. For the analysis, the procedure lme in R (version 2.1) was used (39). Results are presented as estimate (95% CI), unless otherwise stated.

TABLE 1
Clinical characteristics of the study patients

Characteristics	
n	9
Age (years)	52.3 ± 2.9
HIV infection duration (years)	16.3
LogHIV RNA (copies/ml)	1.90 ± 0.55
CD4 + cell counts (cells/μl)	732 ± 118
HAART combination	
PI, NRTI	3
PI, NRTI, NNRTI	4
NRTI, NNRTI	1
NRTI	1
Body weight (kg)	81.9 ± 3.2
BMI (kg/m^2)	25.0 ± 0.8
Body fat (%)	19.9 ± 2.2
Fat mass (kg)	16.8 ± 2.4
Lean body mass (kg)	62.5 ± 12.7
Truncal fat mass (kg)	10.4 ± 1.6
Limb fat mass (kg)	5.5 ± 0.95
Trunk-to-limb ratio	2.00 ± 0.36
Fasting glucose (mmol/l)	5.5 ± 0.23
Fasting insulin (pmol/l)	86 ± 18
Fasting triglycerides (mmol/l)	3.07 ± 0.74
Fasting total cholesterol (mmol/l)	6.1 ± 0.48
Fasting HDL cholesterol (mmol/l)	1.4 ± 0.35
Fasting LDL cholesterol (mmol/l)	3.73 ± 0.74
Fasting FFAs (μmol/l)	412 ± 61.3

Data are means ± SE. HAART, highly active antitretroviral therapy: PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor.

RESULTS

Baseline characteristics. Clinical characteristics of subjects are shown in Table 1. All subjects had lipodystrophy, as indicated by their trunk-to-limb fat ratio, elevated triglycerides (plasma triglycerides >1.7 mmol/l) (40), and plasma FFA concentration (plasma FFAs in healthy men 292 ± 58 μmol/l) (41). All patients received at least one nucleoside analogue, seven received at least one protease inhibitor, and five a nonnucleoside analogue. All patients except one had suppressed HIV RNA.

Effect of acipimox on metabolic biochemistry. Basal plasma glucose and insulin concentrations were not different in the placebo and acipimox trials (Fig. 2*A* and *B*).

Basal plasma FFA concentration was 51.6% (95% CI 43.0–58.9) lower during the acipimox than during the placebo trial. During stage 1 of the clamp, plasma FFA concentration was reduced 61.3% (53.4–67.8) in the acipimox trial compared with a 46.2% (35.9–54.9) reduction in

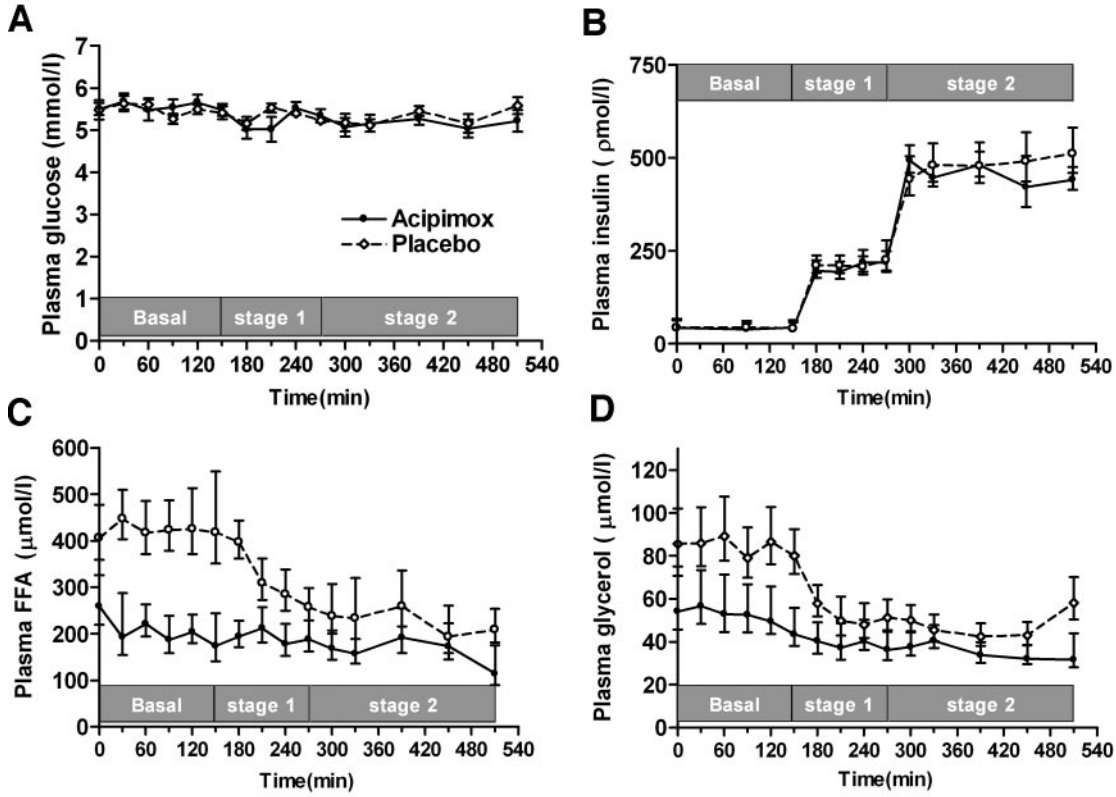

FIG. 2. Plasma glucose (*A*), insulin (*B*), FFAs (*C*), and glycerol (*D*) levels during the basal stage, low-dose insulin infusion (stage 1), and high-dose insulin infusion (stage 2) after overnight treatment with placebo (○) and acipimox (●). Data are presented as means ± SE for glucose and as geometric means ± geometric SE for insulin, FFAs, and glycerol.

the placebo trial. During stage 2, the clamp plasma FFA concentrations were not reduced further ($P < 0.0001$ for treatment effect, $P < 0.0001$ for low-dose insulin effect during placebo, $P = 0.035$ for low-dose insulin effect during acipimox, and $P = 0.27$ for high-dose insulin effect) (Fig. 2*C*).

Basal plasma glycerol concentration was 36.5% (95% CI 29.2–43.1) lower during the acipimox than during the placebo trial. During stage 1, plasma glycerol concentration was reduced 58.7% (53.6–63.3) in the acipimox trial compared with a 43.4% (36.3–49.7) reduction in the placebo trial. During stage 2, plasma glycerol concentrations were not reduced further ($P < 0.0001$ for treatment effect, low-dose insulin effect during placebo, and low-dose insulin effect during acipimox and $P = 0.89$ for high-dose insulin effect) (Fig. 2*D*).

Effect of acipimox on palmitate R_a. Palmitate R_a decreased by 68.9% (95% CI 52.6–79.5) in the basal stage in the acipimox trial compared with placebo (acipimox: 0.65 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [95% CI 0.29–1.45] versus placebo: 2.08 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [1.41- 3.06]; $P < 0.0001$). The decrease in palmitate R_a during stage 1 was greater during acipimox treatment compared with placebo (acipimox: 0.42 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [0.18- 0.95] versus placebo: 0.74 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [0.32- 1.70] or 79.9 vs. 64.6% compared with baseline; $P = 0.0086$). Palmitate R_a did not differ significantly between the acipimox and placebo trials during stage 2 (acipimox: 0.31 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [0.14- 0.71] versus placebo: 0.39 [0.17–0.9] or 85.0 vs. 81.1% compared with baseline; $P = 0.27$). The effect of insulin infusion was significant for both the acipimox ($P = 0.002$) and the placebo ($P < 0.0001$) trials (Fig. 3).

Effect of acipimox on glucose R_a and R_d. During basal conditions, glucose R_a and glucose R_d were the same for the placebo and the acipimox trials (acipimox: 14.77 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [95% CI 8.85–24.66] versus placebo: 14.29 $\mu mol \cdot kg^{-1} \cdot min^{-1}$[11.38–17.93]). Glucose R_a was also not influenced by acipimox treatment during the clamp (stage 1: acipimox: 8.53 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [5.06–14.40] versus placebo: 7.78 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ [4.61–13.12] or 41.3 vs.

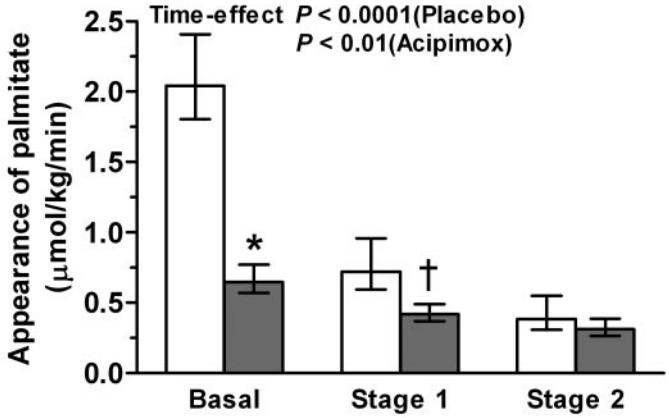

FIG. 3. Histogram shows effect of treatment (placebo [□] versus acipimox [■]) and time (insulin) on palmitate rate of appearance. Basal = no insulin infusion, clamp stage 1 = 20 mU/m² per min insulin infusion, and clamp stage 2 = 50 mU/m² per min insulin infusion. *$P < 0.0001$, placebo vs. acipimox during basal stage; †$P < 0.01$, placebo vs. acipimox during clamp stage 1. Time effect = the effect of insulin for both placebo treatment and acipimox treatment. Values are geometric means ± geometric SE.

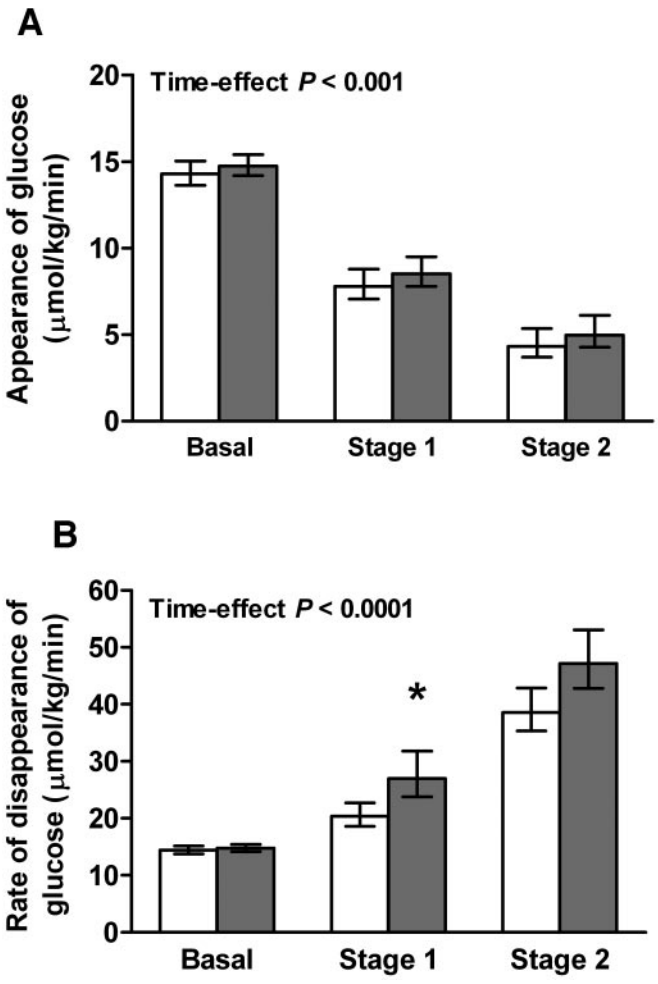

FIG. 4. Histograms show effect of treatment (placebo [□]) versus acipimox [■]) and time (insulin) on glucose rate of appearance (*A*) and disappearance (*B*). Basal = no insulin infusion, clamp stage 1 = 20 mU/m^2 per min insulin infusion, and clamp stage 2 = 50 mU/m^2 per min insulin infusion. **P* = 0.01, placebo vs. acipimox during clamp stage 1. Time effect = the effect of insulin for placebo treatment and acipimox treatment. Values are geometric means ± geometric SE. Glucose rates of appearance and disappearance are measured as μmol · kg body wt^{-1} · min^{-1}.

46.5% compared with baseline. Stage 2: acipimox: 4.96 μmol · kg^{-1} · min^{-1} [2.94–8.37] versus placebo: 4.40 μmol · kg^{-1} · min^{-1} [2.58–7.51] or 65.9 vs. 69.7% compared with baseline). The effect of insulin infusion on R_a was significant for both the acipimox and the placebo trials (P = 0.0003) (Fig. 4*A*).

Glucose R_d increased to a greater extent in the acipimox than in the placebo trial during stage 1 (acipimox: 26.85 μmol · kg^{-1} · min^{-1} [95% CI 18.09–39.86] versus placebo: 20.30 μmol · kg^{-1} · min^{-1} [13.67–30.13] or 84.1 vs. 39.2% compared with baseline; $P < 0.01$). Glucose R_d did not differ significantly in the acipimox and placebo trials during high-dose insulin infusion (acipimox: 47.00 μmol · kg^{-1} · min^{-1} [31.66–69.77] versus placebo: 40.57 μmol · kg^{-1} · min^{-1} [27.09–60.77] or 222.2 vs. 178.3% compared with baseline; $P = 0.16$). The effect of insulin infusion was significant for both the acipimox and the placebo trials ($P < 0.0001$) (Fig. 4*B*).

Effects of acipimox on regulation of glycogen synthase activity and insulin signaling. Both low and high insulin infusion increased GS activity (I-form) ($P <$ 0.0001), decreased phosphorylation on GS site 3a + b ($P <$ 0.01), and increased phosphorylation on both GSK-3β-Ser^9 ($P < 0.001$) and Akt Thr^{308} ($P < 0.0001$) (Fig. 5*A–D*) measured in muscle tissue preparations. In response to acipimox treatment, phosphorylation of GS site 3a + b decreased (treatment effect, $P = 0.017$) (Fig. 5*B*) and the percent I-form activity of GS increased (treatment effect, $P = 0.01$ [log transformed] and $P = 0.04$ [nontransformed]) (Fig. 5*A*). Phosphorylation of GSK-3β and Akt did not change in response to acipimox treatment (GSK-3β: $P =$ 0.06 [nontransformed]; $P = 0.05$ [log transformed] and Akt Thr^{308}: $P = 0.42$) (Fig. 5*C* and *D*, respectively). Total GS activity was unchanged by both treatment and insulin (data not shown). The effect of acipimox on R_d appeared partly explained by increased dephosphorylation of GS 3a + b, as 24% of the effect of GS site 3a + b on R_d was due to acipimox treatment.

DISCUSSION

The present study demonstrates that decreasing plasma FFA concentrations and R_a palmitate by acipimox treatment increase peripheral insulin–stimulated glucose uptake in HIV lipodystrophy without any influence on hepatic glucose production.

Similar to the observation of Hadigan et al. (12), who treated patients with HIV lipodystrophy with acipimox for 3 months, we found a decrease of ~50% in basal levels of circulating FFAs and R_a FFAs and an ~30% increase in glucose uptake in response to acipimox. The fact that acute and chronic acipimox treatment elicited the same responses with regard to plasma FFA and insulin sensitivity suggests that chronic lipid-induced insulin resistance can be reversed equally by acute and chronic intervention. The present study adds to former studies by pinpointing the effect specifically to glucose uptake and on muscle insulin signaling. During stage 1 (low insulin doses), there was a significant difference between the placebo and the acipimox trials with regard to plasma FFAs, R_a palmitate, and R_d glucose. During stage 2 (high insulin dose), lipolysis was totally blocked by insulin, which is most likely the reason for no detectable effect of acipimox on glucose metabolism. The response of R_a FFAs to acute acipimox during high-dose insulin is similar to the response of R_a FFAs to chronic acipimox (12).

It is, to some extent, surprising that we did not find any effect of acipimox on hepatic glucose production, even during low insulin dose. This finding conflicts with some previous observations (31,42,43) using acipimox to suppress lipolysis. Three other studies have demonstrated that acute acipimox treatment decreases basal hepatic glucose (31) and increases hepatic sensitivity to insulin in type 2 diabetic subjects (31,42,43). One explanation for the discrepancy could be that the basal levels of plasma FFAs were higher in the diabetic patients. Taken together, our findings suggest that the nature of hepatic insulin resistance in patients with nondiabetic HIV lipodystrophy may, to some extent, differ from that seen in type 2 diabetes. It is still unknown whether increased hepatic glucose production in HIV lipodystrophy is a result of increased gluconeogenesis or increased glycogenolysis or a combination of both. It could be argued that the patients we selected for the present study did not demonstrate hepatic insulin resistance. However, the subjects had increased R_a glucose (8.1 μmol · kg body wt^{-1} · min^{-1}; ~6.2 μmol · kg fat-free mass^{-1} · min^{-1}) compared with R_a glucose in healthy men (~2.1 μmol · kg fat-free mass^{-1} · min^{-1})

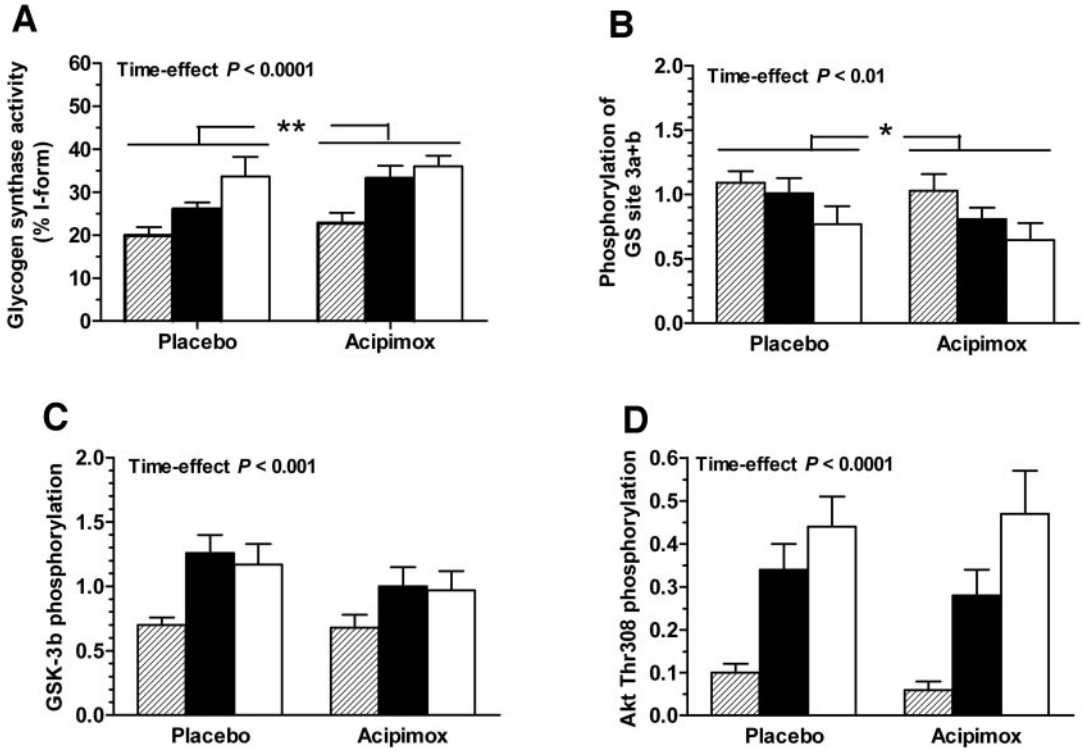

FIG. 5. Histograms show effect of treatment (placebo versus acipimox) and time (insulin) on glycogen synthase activity given as percent I-form activity (*A*) and on phosphorylation of GS at the COOH-terminal site 3a + b (*B*), of GSK-3β Ser^{9} (*C*), and of Akt Thr^{308} (*D*) in skeletal muscle. Basal (▨) = no insulin infusion, clamp stage 1 (■) = 20 mU insulin infusion, and clamp stage 2 (□) = 50 mU insulin infusion. Placebo: n = 8, 7, 8; and Acipimox: n = 7, 6, 6 for basal, clamp stage 1, and clamp stage 2, respectively. *P < 0.05; ** P < 0.01. Time effect = the effect of insulin for both placebo treatment and acipimox treatment. Values are means ± SE.

during low insulin dose (3). Inhibition of insulin-stimulated glucose uptake by FFAs is supported by other studies, including experimental designs, where plasma FFA levels were lowered by acipimox (19,30,44,45) or increased by lipid infusions (14,46–48). In these studies, the effect on glucose uptake was observed at a high insulin dose (40–80 mU/m^2 per min). Our study shows that the effect of suppression of FFA levels may, in fact, be more easily detected at lower insulin concentrations (20 mU/m^2 per min).

Patients with HIV lipodystrophy are characterized by having decreased oxidative and nonoxidative glucose disposal (25–27). During insulin stimulation, nonoxidative glucose metabolism is thought to primarily represent muscle glycogen synthesis (49). As expected, insulin administration induced increased phosphorylation of Akt Thr^{308} and GSK-3β Ser^{9}, decreased phosphorylation on GS (site 3a + b), and subsequently increased the activity of GS in both trials. Interestingly, in the response to acipimox treatment, GS activity (%I-form) was greater and phosphorylation of GS (site 3a + b) decreased compared with before treatment. Moreover, acipimox-induced increase in glucose uptake was associated with acipimox-induced improvement of the insulin signaling pathway, although this does not prove causality.

Regulation of GS activity by manipulating FFA concentration is in accordance with previous studies (13–15,17–19). GSK-3 is a serine/threonine kinase that inhibits GS activity by phosphorylation of GS (50) at sites 4, 3c, 3b, and 3a (51). However, in our study, we did not find a significant effect of acipimox treatment on phosporylation of GSK-3β Ser^{9}. This finding is surprising but seems consistent with the observation in rodents that insulin-stimulated glycogen synthase activity is inhibited by infusion of lipids independent of deactivation of GSK-3 (24). In addition, Skurat et al. (50) have previously, in an in vitro study, demonstrated that GS (site 3a + b) can be phosphorylated by unidentified protein kinases, suggesting regulation of GS activity by non–GSK-3–mediated pathways (24,52).

Upstream of GSK-3, acipimox treatment correspondingly did not effect phosphorylation of Akt Thr^{308}, a marker of Akt activity. Conflicting data on regulation of phosphorylation of Akt by lipids exist. In a study by Kryszynska et al. (23), intralipid infusion did not change phosphorylation of Akt Thr^{308} and Akt Ser^{473} despite reduced whole-body insulin-stimulated glucose disposal. In contrast, two other in vivo studies (humans [21] and rats [24]) find impaired phosphorylation of Akt Thr^{308} and Ser^{473} in response to lipid infusion. These discrepancies may indicate that the signaling mechanism is different when inducing acute insulin resistance by lipid infusion compared with reversing chronic insulin resistance by acipimox treatment. Furthermore, it cannot be excluded that acipimox itself may directly stimulate glycogen synthesis in skeletal muscle, although no receptors for nicotinic acid in skeletal muscle have been identified (53). Finally, it is possible that acipimox treatment may lead to improved activation of protein phosphatases regulating GS activity during insulin stimulation. This, however, remains to be investigated.

The present study provides direct evidence that suppression of lipolysis in patients with HIV lipodystrophy improves insulin-stimulated peripheral glucose uptake without influencing hepatic insulin resistance. The increased glucose uptake may in part be explained by increased dephosphorylation of GS (site 3a + 3b), result-

ing in increased GS activity independently of phosphorylation of Akt Thr^{308} and GSK-3β Ser^{9}.

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